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Studies on the Constituents of *Aloe saponaria* Haw. II.[1] The Structures of Tetrahydroanthracene Derivatives, Aloesaponol III and -IV

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Phenolic constituents of young subterranean stems of *Aloe saponaria* Haw. were investigated to give aloesaponol III, -IV, chrysophanol, helminthosporin and 1,4,8-trihydroxy-2-methoxy-6-methyl-anthraquinone(isoxanthorin).

The structures of aloesaponol III and -IV were established to be 1-oxo-4(*S*, equatorial), 8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene and 1-oxo-4(quasi-equatorial), 8,9-trihydroxy-2-methoxy(equatorial)-6-methyl-1,2,3,4-tetrahydroanthracene on the basis of chemical and spectral evidences.

The application of the extended benzoate chirality rule was attempted to determine the absolute configuration of hydroxyl group in aloesaponol I and -III.

Keywords——*Aloe saponaria* Haw.; Liliaceae; young subterranean stems; structures of aloesaponol III and -IV; new tetrahydroanthracene derivatives; absolute configurations of aloesaponol I and -III

In the preceding paper, we reported isolation and structure elucidation of tetrahydroanthracene pigments, aloesaponol I, -II and the related anthraquinones from fresh young subterranean stems of *Aloe saponaria* Haw.[1] Further examination on phenolics of this plant was carried out to afford tetrahydroanthracene derivatives, named aloesaponol III and -IV, along with the related anthraquinones. This paper deals with structure elucidation of aloesaponol III and -IV and the elucidation of the absolute configuration of C_4 hydroxyl group in aloesaponol III and of C_3 hydroxyl group in aloesaponol I.

The fresh chips of the plant were treated as described in the experimental section to yield aloesaponol III, -IV, chrysophanol, helminthosporin, and 1,4,8-trihydroxy-2-methoxy-6-methyl-anthraquinone (isoxanthorin).

A yellow pigment, aloesaponol III, mp 198—200°, $[\alpha]_D^{18}$ +20° (acetone), $C_{15}H_{14}O_4$, giving positive Gibbs' and alkali tests, exhibited similar ultraviolet (UV) (λ_{max}^{MeOH} nm: 270, 298, 324, 405) and infrared (IR) (ν_{max}^{KBr} cm^{-1}: 1637) spectra to those of germichrysone.[3] Nuclear magnetic resonance (NMR) spectrum of aloesaponol III revealed a multiplet methylene proton (C_2) signal at δ 2.30, a methylene proton (C_3) signal at δ 2.75 (J=18; 7; 6) and 3.05 (J=18; 6.5; 6), a methyl proton signal at δ 2.48 and a methine proton (C_4) signal at δ 4.94 (J=6; 4). Of aromatic protons two *meta* coupling proton (C_5 and C_7) signals appeared at δ 6.76 and 7.02, and a singlet proton (C_{10}) signal at δ 7.14. Two phenolic and an alcoholic hydroxyl proton signals appeared at δ 9.68, 16.02 and 1.80.

Aloesaponol III was dehydrated with diluted HCl–MeOH to the anhydro compound,[4] which without isolation was converted to chrysophanol by successive air oxidation in an alkaline solution.

On acetylation, aloesaponol III gave diacetate, mp 115—117°, $C_{19}H_{18}O_6$, which indicated a bathochromic shift in the UV spectrum when $AlCl_3$ was added to the solution and a chelated

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2) Location: *Maidashi, Higashi-ku, Fukuoka.*
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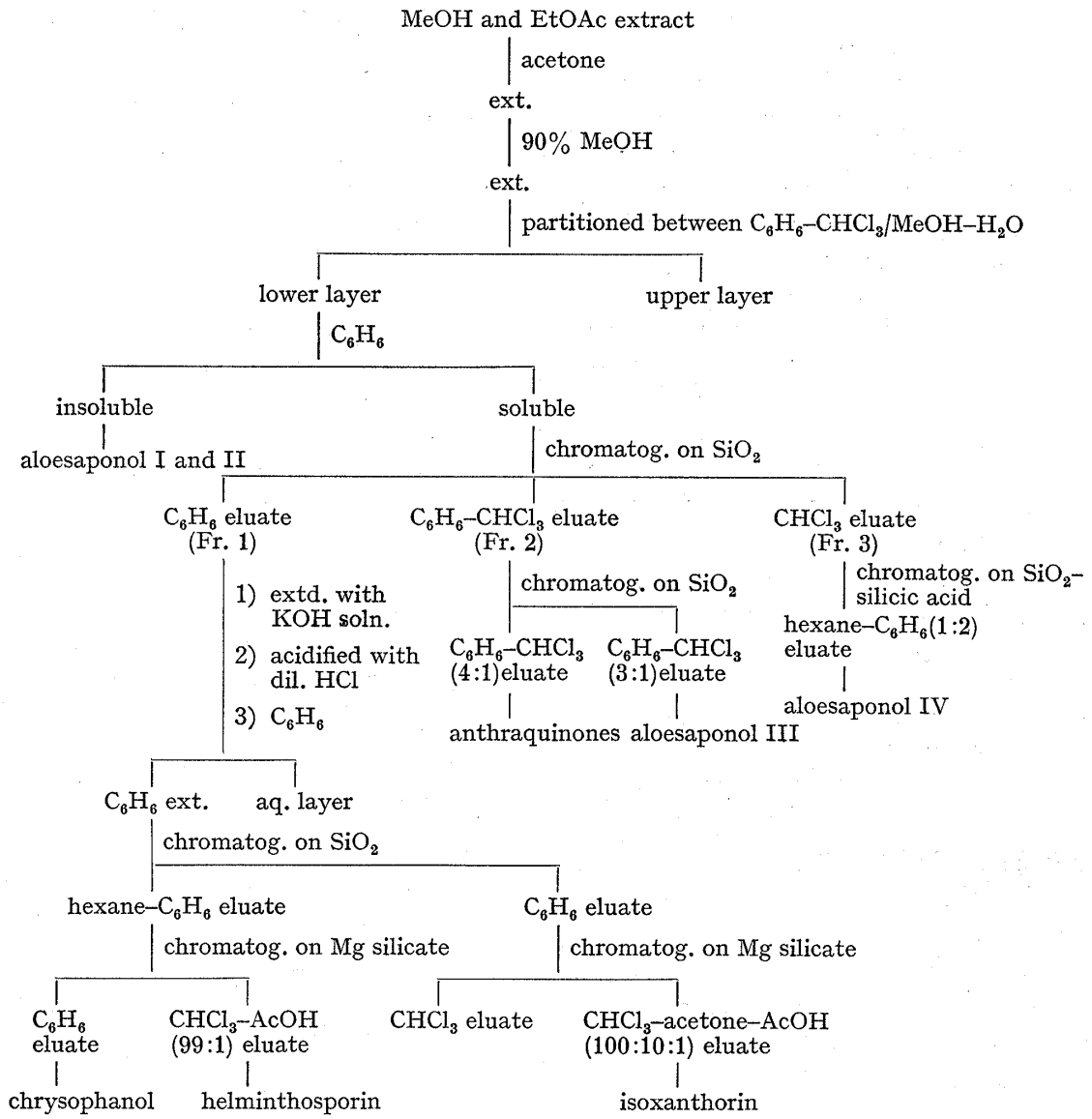
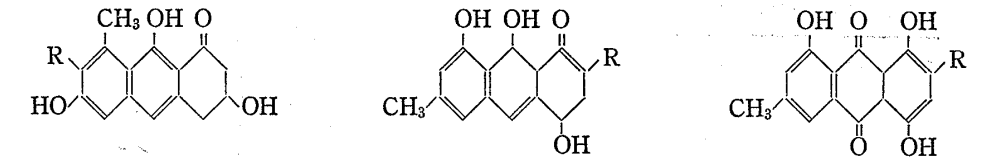


Chart 1



aloesaponol I: R=$COOCH_3$
aloesaponol II: R=H
aloesaponol III: R=H
aloesaponol IV: R=OCH_3
helminthosporin: R=H
isoxanthorin: R=OCH_3

Chart 2

carbonyl absorption band in the IR spectrum. On the NMR spectrum, the diacetate showed two acetoxy methyl proton signals at δ 2.07 and 2.34 and a methyl proton signal at δ 2.52. Two methylene (C_3 and C_2) and a methine proton (C_4, t, J=4.5) signals appeared at δ 2.30, 2.70—2.95 and 6.06, respectively, and a hydrogen-bonded phenolic proton (C_9) at δ14.80. Of aromatic protons two *meta* coupling protons located at *ortho* or *para* position of C_8-OAc, and a singlet proton (C_{10}) signals appeared at δ 6.85, 7.38 and 7.15, respectively. Further-

more, a long range coupling between C_{10}-H (δ 7.15) and C_4-H (δ 6.06) was confirmed by a spin decoupling experiment.[5]

On the basis of the above evidence it is reasonably suggested that an alcoholic group is located at C_4 in 1-oxo-2(or 4), 8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene. This was confirmed by the fact that on oxidation with MnO_2 aloesaponol III provided helminthosporin[6] which was isolated from this plant. The conformation of C_4 hydroxyl group was assigned to be quasi-equatorial by examination of the coupling constant($J_{aa}=6$; $J_{ae}=4$)between C_4-methine and C_3-methylene protons.[7]

Accordingly, the structure of aloesaponol III was demonstrated to be 1-oxo-4(quasi-equatorial),8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene.

The absolute configuration of C_4 hydroxyl group in aloesaponol III was established by the application of the extended benzoate chirality rule.[5,8]

On methylation with CH_2N_2 aloesaponol III afforded two monomethyl ethers A, mp 173—175°, $C_{16}H_{16}O_4$ and B, mp 70—71°, $C_{16}H_{16}O_4$. Since monomethyl ether A showed a hydrogen-bonded hydroxyl proton signal on the NMR spectrum (δ 15.16) and a chelated carbonyl absorption band on the IR spectrum (ν_{max}^{KBr} cm^{-1}: 1625), the structure was proved to be C_8-monomethyl ether of aloesaponol III.

Conventional *p*-bromobenzoylation of C_8-monomethyl ether yielded mono-*p*-bromobenzoate, mp 121—122°, $C_{23}H_{19}O_5Br$. The NMR spectrum indicated a methyl proton (C_6) signal at δ 2.44, signals of two methylene protons at δ 2.76 (C_2, $J=18$; 6; 5.5), δ 3.04 (C_2, $J=18$; 7; 6.5) and 2.3—2.5 (C_3), a methoxy proton (C_8) signal at δ 3.93, and a methine proton (C_4) signal at δ 6.26 (t, $J=4.5$).[7] Of aromatic protons two doublet proton (C_5 and C_7) signals appeared at δ 6.56 and 6.96 ($J=1.5$), a singlet proton (C_{10}) signal at δ 7.00 and two A_2B_2 system proton signals due to *p*-bromobenzoate ($C_{3'}$, $C_{5'}$ and $C_{2'}$, $C_{6'}$) at δ 7.53 and 7.88, and a hydroxy proton (C_9) signal at δ 14.76. Accordingly, it was verified that C_4 hydroxyl group in *p*-bromobenzoate has the same conformation as in aloesaponol III. Both optical rotatory dispersion (ORD) and circular dichroism (CD) curves of *p*-bromobenzoate indicated the positive Cotton effects, ORD ($c=0.0008$, cyclohexane): $[\phi]^{p}_{266}$ $+6.3\times10^4$, $[\phi]^{t}_{251}$ -5.2×10^3; CD ($c=0.008$, cyclohexane): $[\theta]^{p}_{259}$ $+7.5\times10^4$, $[\theta]^{t}_{230}$ -3.8×10^4, while aloesaponol III exhibited only a simple Cotton effect. The first positive Cotton effect indicates that the *p*-bromobenzoate has a positive chirality, and the absolute configuration of C_4 hydroxyl group in aloesaponol III is assigned to S.

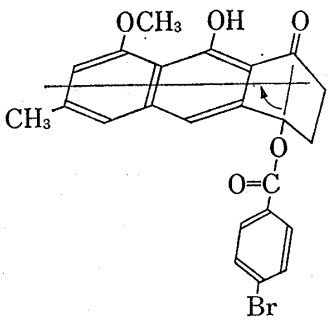


Fig. 1. The Positive Chirality of 4-*O*-*p*-Bromobenzoyl-8-*O*-methyl Aloesaponol III

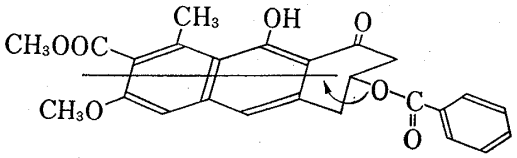


Fig. 2. The Positive Chirality of 3-*O*-Benzoyl-6-*O*-methyl-aloesaponol I

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A red pigment, isoxanthorin, mp 236—239°, $C_{16}H_{12}O_6$, indicated characteristic UV and IR spectra of 1,4,8-trihydroxyanthraquinone.[6] The NMR spectrum exhibited a methoxy proton signal at δ 4.00 and three hydrogen-bonded hydroxyl protons at δ 12.02, 12.74, and 13.60. Of aromatic protons two *meta* coupling proton signals which showed a long range coupling with a methyl proton at δ 2.49 appeared at δ 7.07 and 7.68 and a singlet proton at δ 6.68. isoxanthorin the position of a methoxyl group was assumed to be at C_2 or C_3. The structure of Therefore, was distinctly confirmed to be 1,4,8-trihydroxy-2-methoxy-6-methyl-anthraquinone by careful comparative studies on the spectral data with those of xanthorin[9] which was synthesized from emodin.

A yellow pigment, aloesaponol IV, mp 197—200° (decomp.), $[\alpha]_D^{26}$ −11.6° ($CHCl_3$), $C_{16}H_{16}O_5$, showed the same coloration to those of aloesaponol III and presented characteristic absorption bands due to 1-oxo-1,2,3,4-tetrahydroanthracene skeleton on the UV (λ_{max}^{MeOH} nm: 271, 310, 405) and the IR (ν_{max}^{KBr} cm^{-1}: 1637) spectra. On the NMR spectrum aloesaponol IV indicated a multiplet methylene proton (C_3) signal at δ 2.2—2.8, a methyl proton (C_6) signal at δ 2.42, a methoxy proton signal at δ 3.64, two methine proton (C_2 and C_4) signals at δ 4.38 and 5.06. Of aromatic protons two *meta* coupling (C_5 and C_7) and a singlet (C_{10}) proton signals appeared at δ 6.71, 6.92 and 7.06, respectively, and two phenolic proton signals at δ 9.51 and 15.70. The assignments of the methylene and two methine protons were confirmed by spin decoupling experiments. Irradiation at δ 2.40 (C_3-H) collapsed two double doublets at C_2-H (δ 4.38) and C_4-H (δ 5.06) to two singlets, and a long range coupling between C_4-H (δ 5.06) and C_{10} (δ 7.06) was demonstrated. On aerial oxidation aloesaponol IV afforded isoxanthorin which was isolated from this plant.

Therefore, the position of the methylene was established to be C_3, and a hydroxyl and a methoxyl groups were located at C_4 and C_2, respectively.

The conformation of methine protons at C_2 and C_4 in aloesaponol IV was assigned to be axial and quasi-axial, respectively by examination of the coupling constant between C_2-H and C_3-H (C_2-H, J_{aa}=8; J_{ae}=4), and C_4-H and C_3-H (C_4-H, J_{aa}=6; J_{ae}=4).[7] Therefore, the structure of aloesaponol IV was established to be 1-oxo-4(quasi-equatorial), 8,9-trihydroxy-2-methoxy(equatorial)-6-methyl-1,2,3,4-tetrahydroanthracene.

The absolute configuration of the C_3 hydroxyl group in aloesaponol I was determined as follows. Benzoylation of 6-*O*-methyl-aloesaponol I with dimethylaniline and C_6H_5COCl gave 3-*O*-benzoyl-6-*O*-methyl-aloesaponol I. The benzoate, mp 157—160°, $C_{25}H_{22}O_7$, gave a positive $FeCl_3$ test and showed the similar UV and IR spectra to those of 6-*O*-methyl-aloesaponol I. The NMR spectrum indicated two methylene proton signals at δ 3.07 (C_2-H, J_{gem}=16; J_{aa}=7), 3.20 (C_2-H, J_{gem}=16; J_{ea}=4), 3.30 (C_4-H, J_{gem}=16; J_{aa}=7), 3.42 (C_4-H, J_{gem}=16; J_{ea}=4), a methine proton signal (C_3-H, m, $W/2$=16) at δ 5.70 besides the proton signals at δ 2.88 (C_8-CH_3), 3.92 and 3.98 (C_7-CH_3COO or C_6-CH_3O), 6.83 (C_5-H), 6.94 (C_{10}-H), 7.3—7.6 ($C_{3'}$, $C_{4'}$, $C_{5'}$-H), 7.94 ($C_{2'}$, $C_{6'}$-H, J=8; 2) and 14.94 (C_9-OH). In confirmation of the assignments of two methylene (C_2 and C_4) and a methine (C_3) protons, irradiation at δ 3.30 collapsed the multiplet at δ 5.70 ($W/2$=16) to broad singlet and irradiation at δ 5.70 collapsed double doublets at δ 3.07, 3.20, 3.30 and 3.42 to four doublets (J=16). The NMR inspection and the spin decoupling experiments disclosed that this benzoate existed in the same conformation as aloesaponol I in which C_3 hydroxyl group was assigned to be equatorial.[10]

Both ORD and CD curves of the benzoate showed the positive Cotton effect ORD (c=0.001, MeOH): $[\phi]_{274}^{p}$ $+3.2\times10^4$, $[\phi]_{240}^{t}$ -4.8×10^4; ORD (c=0.0009, cyclohexane): $[\phi]_{275}^{p}$

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10) The NMR spectrum of aloesaponol I in pyridine δ: 3.12 (3H, s, CH_3), 3.11 (1H, dd, J_{gem}=16; J_{aa}=7, C_2–H), 3.22 (1H, dd, J_{gem}=16; J_{ae}=4, C_2–H), 3.20 (1H, dd, J_{gem}=16; J_{aa}=7, C_4–H), 3.32 (1H ,dd, J_{gem}=16; J_{ae}=4, C_4–H), 4.60 (1H, m, $W/2$=16, C_3–H), 6.84 (1H, s, C_5 or C_{10}–H), 7.19 (1H, s, C_5 or C_{10}–H).

$+2.9\times10^4$, $[\phi]^{t}_{240}$ -3.7×10^4; CD (c=0.01, MeOH): $[\theta]^{p}_{270}$ $+1.0\times10^5$, $[\theta]^{t}_{220}$ -5.0×10^4, CD (c=0.009, cyclohexane): $[\theta]^{p}_{272}$ $+5.1\times10^4$, $[\theta]^{t}_{232}$ -7.0×10^4, while 6-*O*-methyl-aloesaponol I exhibited only a simple Cotton effect.

Consequently, it is indicated that the benzoate has a positive chirality and the absolute configuration at C_3 hydroxyl group in aloesaponol I is assigned to R.

On a biogenetic point of view, the presence of aloesaponol III, -IV, helminthosporin and isoxanthorin, in which a hydroxyl groups was located at C_4 is of significance.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and uncorrected. IR spectra were obtained with a KOKEN DS-301 and UV spectra were recorded with a Shimadzu SV-50A. NMR spectra were taken with a JEOL-100H spectrometer. Chemical shifts were expressed in ppm from Me_4Si as internal reference and coupling constant (J) in Hz. Abbreviation used, s=singlet, d=doublet, t=triplet, oct=octet, m=multiplet, dd=double doublet, br=broad. Mass spectra were determined on a JEOL-10 double forcus high resolution spectrometer. ORD and CD spectra were measured on a JASCO ORD/UV 5 spectrometer at 25°, and optical rotation on JASCO DIP-SL automatic polarimeter. TLC were performed on silica gel G (Merck) employing the following solvent systems. TLC I: C_6H_6: acetone (20: 1), TLC II: hexane: C_6H_6: AcOH (20: 15: 1) for anthraquinones; TLC III: EtOAc: MeOH: H_2O (50: 1: 1) for tetrahydroanthracenes. As a spraying reagent 10% KOH–MeOH solution was used and as a monitor UV lamp (PUV-1B, Kogaku Kikai) was used. Column chromatography was carried out on silica gel (Kiesel gel 60, 70—230 mesh, Merck, or silicic acid, 100 mesh, Mallinckrodt) and $MgSiO_3$ (Woelm, activity grade 1).

Unless otherwise specified, silica gel used was the product of Merck.

Isolation of Phenolics——The fresh young chips (10 kg) of subterranean stems of this plant were successively extracted with MeOH (5 l) and EtOAc (5 l) four times, respectively. To the combined extracts (340 g) acetone (2 l) was added and the filtrate was concentrated to syrup. The syrup was treated with 90% MeOH and the MeOH extract (45 g) was partitioned between $CHCl_3$–C_6H_6 (2: 1) and MeOH–H_2O (1: 3). The lower layer was evaporated to dryness (5 g) and the C_6H_6 soluble portion (2.8 g) was chromatographed over silica gel using C_6H_6 (Fr. 1), C_6H_6–$CHCl_3$ (Fr. 2) and $CHCl_3$ (Fr. 3), successively, as solvents. In order to extract phenolics the C_6H_6 eluate (Fr. 1) was treated with 3% KOH and after the acidification of the solution, phenolics were extracted with C_6H_6. The C_6H_6 extract was chromatographed over silica gel using hexane–C_6H_6 and C_6H_6 as solvents. The hexane–C_6H_6 eluate was subjected to chromatography on $MgSiO_3$ using C_6H_6 and $CHCl_3$–AcOH (99: 1) as solvents to give chrysophanol and helminthosporin. The C_6H_6 eluate was chromatographed on $MgSiO_3$ using $CHCl_3$ and $CHCl_3$–acetone–AcOH (100: 10: 1) as solvents to give isoxanthorin. The C_6H_6–$CHCl_3$ (1: 1) eluate (Fr. 2) was chromatographed over silica gel using C_6H_6–$CHCl_3$ (3: 1) as solvent to give aloesaponol III (20 mg). The $CHCl_3$ eluate (Fr. 3) was chromatographed on silica gel (Kiesel gel 60, Merck) and silicic acid (Mallinckrodt) (1: 2) using hexane–C_6H_6 (1: 2) as solvent to give aloesaponol IV (12 mg).

Chrysophanol——mp 197°, orange needles (recrystallized from acetone), UV λ^{MeOH}_{max} nm: 226, 256, 278, 287, 430; IR ν^{KBr}_{max} cm^{-1}: 1675, 1630, 1610; MS m/e: 254 (M^+).

Helminthosporin——mp 210—212°, red needles (recrystallized from ether), UV λ^{MeOH}_{max} nm: 230, 253, 287, 298, 460, 476, 489, 507, 522, 567; $\lambda^{MeOH\text{-}KOH}_{max}$ nm: 239, 270, 281, 290, 510, 520, 540, 585; IR $\nu^{CCl_4}_{max}$ cm^{-1}: 1603; MS m/e: 270.052 (M^+, Calcd. for $C_{15}H_{10}O_5$ 270.053), 254; NMR ($CDCl_3$) δ: 2.50 (3H, s, CH_3), 7.10 (1H, d, J=1.5, C_7–H), 7.28 (2H, s, C_2, C_3–H), 7.68 (1H, d, J=1.5, C_5–H), 12.08, 12.26, 12.96 (3H, each, OH).

Isoxanthorin——mp 236—239°, red needles (recrystallized from ether), MS m/e: 300.065 (M^+, Calcd. for $C_{16}H_{12}O_6$, 300.063), 282; UV λ^{MeOH}_{max} nm (log ε): 233 (4.4), 250 (4.5), 302 (3.9), 312 (3.8), 458 (3.8), 486 (4.0), 519 (4.1), 560 (3.9); IR ν^{KBr}_{max} cm^{-1}: 1600; NMR ($CDCl_3$) δ: 2.49 (3H, s, CH_3), 4.00 (3H, s, CH_3O), 6.68 (1H, s, C_3–H), 7.07 (1H, d, J=2, C_7–H), 7.68 (1H, d, J=2, C_5–H), 12.02, 12.74, 13.60 (3H, each, OH).

Synthesis of Xanthorin——The solution of emodin (200 mg) dissolved in 25% KOH (4 ml) was heated at 150—160° for 6 minutes. The reaction mixture was neutralized with diluted HCl and extracted with ether. The product dissolved in ether was methylated with CH_2N_2 at 0° for 10 minutes and after evaporation of the solvent the residue was chromatographed over silica gel using hexane–C_6H_6 (1: 2) as solvent. Recrystallization from $CHCl_3$ gave xanthorin (80 mg) as red needles, mp 247—248°, UV λ^{MeOH}_{max} nm (log ε): 236 (4.4), 250 (4.4), 257 (4.5), 300 (3.9), 308 (sh.), 460 (4.1), 488 (4.2), 515 (4.0), 523 (4.0), 559 (3.3); IR ν^{KBr}_{max} cm^{-1}: 1603, NMR ($CDCl_3$) δ: 2.48 (3H, s, CH_3), 4.00 (3H, s, CH_3O), 6.65 (1H, s, C_2–H), 7.08 (1H, d, J=1.5, C_7–H), 7.76 (1H, d, J=1.5, C_5–H), 12.22, 12.73, 13.52 (3H, each OH); MS m/e: 300(M^+), 282 (M^+-H_2O).

Aloesaponol III——Recrystallization from ether gave yellow needles, mp 198—200° (decomp.), $[\alpha]^{18}_{D}$ +20° (c=1.25, acetone), MS m/e: 258.085 (M^+, Calcd. for $C_{15}H_{14}O_4$, 258.089), 240 (M^+-H_2O), 229, 225, 212, 202, 201, 197, 173; UV λ^{MeOH}_{max} nm (log ε): 225 (4.4), 262 (sh.), 270 (4.6), 298 (3.5), 309 (3.4), 392 (sh.), 405 (3.8); UV $\lambda^{MeOH\text{-}AlCl_3}_{max}$ nm: 226, 276, 313, 330, 420, 445; IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3400, 1637, 1610, 1580; ν^{KBr}_{max} cm^{-1}: 3520, 3390, 1640, 1618, 1585; NMR ($CDCl_3$) δ: 1.80 (1H, br. s, OH), 2.30 (2H, m, C_3–H), 2.48 (3H, s, CH_3), 2.75 (1H,

oct, J=18; 7; 6, C_2–H), 3.05 (1H, oct, J=18; 6.5; 6, C_2–H), 4.94 (1H, dd, J=6; 4, C_4–H), 6.76 (1H, d, J=2, C_5 or C_7–H), 7.02 (1H, d, J=2, C_5 or C_7–H), 7.14 (1H, s, C_{10}–H), 9.68 (1H, s, C_8–OH), 16.02 (1H, s, C_9–OH).

Conversion of Aloesaponol III to Chrysophanol——A solution of aloesaponol III (2 mg) dissolved in 10% HCl–MeOH (10 ml) was refluxed for 1 hr. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with ether. The ether extract was washed with H_2O and evaporated to dryness. To the residue dissolved in MeOH (2 ml), 2% KOH (10 ml) was added and the reaction mixture was heated for 10 minutes at 80°. The solution was acidified with diluted HCl and was extracted with ether. The ether extract was chromatographed over silica gel using 1% EtOAc–hexane as solvent to give chrysophanol which was identified by direct comparison (TLC, UV and MS) with an authentic sample.

Diacetate——Aloesaponol III (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) at room temperature. After the usual work up the product was chromatographed over silica gel using C_6H_6 as solvent to give diacetate (5 mg). Recrystallization from $CHCl_3$ gave pale yellow needles, mp 115—117°; MS m/e: 342.113 (M^+, Calcd. for $C_{19}H_{18}O_6$, 342.110), 300 (M^+–Ac), 240 (M^+–Ac, –AcOH), 225, 212, 197; UV λ_{max}^{MeOH} nm (log ε): 232 (4.5), 259 (4.5), 267 (4.5), 293 (3.6), 304 (3.6), 316 (3.3), 378 (3.7), 390 (3.6); $\lambda_{max}^{MeOH\text{-}AlCl_3}$ nm: 232, 268, 276, 296, 309, 319, 414, 430; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1773, 1746, 1630, 1580; NMR ($CDCl_3$) δ: 2.07 (3H, s, Ac), 2.34 (3H, s, Ac), 2.30 (2H, m, C_3–H), 2.52 (3H, s, CH_3), 2.70—2.95 (2H, m, C_2–H), 6.06 (1H, t, J=4.5, C_4–H), 6.85 (1H, d, J=2, C_5 or C_7–H), 7.15 (1H, s, C_{10}–H), 7.38 (1H, d, J=2, C_5 or C_7–H), 14.80 (1H, s, OH).

Oxidation of Aloesaponol III to Helminthosporin——To a solution of aloesaponol III (2 mg) in C_6H_6 (6 ml), MnO_2 (20 mg) was added and the reaction mixture was refluxed for 7 hr. After the usual work up the product was purified by preparative TLC (hexane: ether: AcOH; 20: 10: 2) to afford helminthosporin which was identified by direct comparison (TLC, UV and mixed melting point) with an authentic sample.

Monomethyl Ether A and B——Aloesaponol III (42 mg) dissolved in MeOH (39 ml) was methylated with CH_2N_2 at 0° for 1 hr. The product was purified by silica gel chromatography using hexane–ether (7: 3 and 3: 2) as solvent to give pale yellow monomethyl ether A (12 mg) and yellow monomethyl ether B (22 mg). Monomethyl ether A, mp 173—175°, pale yellow needles (recrystallization from ether), MS m/e: 272.105 (M^+, Calcd. for $C_{16}H_{16}O_4$, 272.105), 254 (M^+-H_2O); UV λ_{max}^{MeOH} nm (log ε): 237 (4.6), 258 (sh.), 266 (4.4), 297 (3.8), 306 (3.8), 320 (3.7), 336 (3.6), 390 (3.7), 404 (3.7); $\lambda_{max}^{MeOH\text{-}AlCl_3}$ nm: 237, 265, 274, 307, 322, 334, 428, 444; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3300, 1625, 1575; ν_{max}^{KBr} cm^{-1}: 3400, 1625, 1590; NMR ($CDCl_3$) δ: 2.00 (1H, br. s, OH), 2.28 (2H, m, C_3–H), 2.48 (3H, s, CH_3), 2.70 (1H, oct, J=18; 6; 4, C_2–H), 3.08 (1H, oct, J=18; 7; 6, C_2–H), 4.00 (3H, s, CH_3O), 4.95 (1H, dd, J=5; 4, C_4–H), 6.68 (1H, d, J=2, C_5 or C_7–H), 7.00 (1H, d, J=2, C_5 or C_7–H), 7.09 (1H, s, C_{10}–H), 15.16 (1H, s, OH). Monomethyl ether B, mp 70—71°, yellow needles (recrystallization from ether), MS m/e: 272.101 (M^+, Calcd. for $C_{16}H_{16}O_4$, 272.105), 254 (M^+-H_2O); UV λ_{max}^{MeOH} nm (log ε): 220 (4.4), 266 (4.5), 304 (3.8), 313 (3.8), 327 (sh.), 328 (3.9); $\lambda_{max}^{MeOH\text{-}AlCl_3}$ nm: 220, 266, 304, 313, 327, 378; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3360, 1687, 1639, 1600, 1570; NMR ($CDCl_3$) δ: 1.86 (1H, s, OH), 2.30 (2H, m, C_3–H), 2.46 (3H, s, CH_3), 2.75 (1H, oct, J=17; 6; 4.5, C_2–H), 3.05 (1H, oct, J=17; 6; 5, C_2–H), 4.05 (3H, s, CH_3O), 5.01 (1H, dd, J=5; 4, C_4–H), 6.78 (1H, d, J=2, C_5 or C_7–H), 7.11 (1H, d, J=2, C_5 or C_7–H), 7.62 (1H, s, C_{10}–H), 9.84 (1H, s, OH).

***p*-Bromobenzoate**——To a solution of aloesaponol III-8-monomethyl ether (7 mg) dissolved in pyridine (5 ml) *p*-bromobenzoyl chloride (150 mg) was added and the reaction mixture was stirred for 2 hr at 50°. The product was extracted with C_6H_6 and after evaporation of the solvent the residue was chromatographed over silica gel using C_6H_6–hexane (1: 1) as solvent. The crude product was purified by preparative TLC (C_6H_6: acetone, 20: 1) followed by recrystallization from MeOH to give yellow needles of *p*-bromobenzoate (8 mg). mp 121—122°, MS m/e: 456.037; 454.044 (M^+, Calcd. for $C_{23}H_{19}O_5Br$, 456.040; 454.042); UV $\lambda_{max}^{cyclohexane}$ nm (log ε): 232 (4.6), 248 (4.6), 253 (4.7), 262 (4.8), 295 (3.4), 305 (3.4), 320 (3.2), 365 (3.8), 383 (4.1), 402 (4.0); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1725, 1625, 1590, 1575; NMR (CCl_4) δ: 2.3—2.5 (2H, m, C_3–H), 2.44 (3H, s, CH_3), 2.76 (1H, oct, J=18; 6; 5.5, C_2–H), 3.04 (1H, oct, J=18; 7; 6.5, C_2–H), 3.93 (3H, s, CH_3O), 6.26 (1H, t, J=4.5, C_4–H), 6.56 (1H, d, J=1.5, C_5 or C_7–H), 6.96 (1H, d, J=1.5, C_5 or C_7–H), 7.00 (1H, s, C_{10}–H), 7.53 (2H, d, J=8, $C_{3'}$, $C_{5'}$–H), 7.88 (2H, d, J=8, $C_{2'}$, $C_{6'}$–H), 14.76 (1H, s, OH).

Aloesaponol IV——Recrystallization from C_6H_6–$CHCl_3$ gave yellow amorphous powder, mp 197—200°, $[\alpha]_D^{26}$ −11.6° (c=0.86, $CHCl_3$); MS m/e: 288.104 (M^+, Calcd. for $C_{16}H_{16}O_5$, 288.100), 270 (M^+-H_2O), 256 (M^+-CH_3OH), 202, 173; UV λ_{max}^{MeOH} nm (log ε): 271 (4.3), 300 (sh.), 310 (3.4), 328 (sh.), 405 (3.8), 420 (sh.); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1637, 1605, 1582; NMR ($CDCl_3$) δ: 2.2—2.8 (2H, m, C_3–H), 2.42 (3H, s, CH_3), 3.64 (3H, s, CH_3O), 4.38 (1H, dd, J=8; 4, C_2–H), 5.06 (1H, dd, J=6; 4, C_4–H), 6.71 (1H, d, J=1.5, C_5 or C_7–H), 6.92 (1H, d, J=1.5, C_5 or C_7–H), 7.06 (1H, s, C_{10}–H), 9.51 (1H, s, OH), 15.70 (1H, s, OH).

Oxidation of Aloesaponol IV to Isoxanthorin——To a solution of aloesaponol IV (1.5 mg) in $CHCl_3$ (1 ml) SiO_2 (100 mg) was added and the mixture was allowed to stand for 3 days at room temperature. The product was chromatographed over silica gel using C_6H_6 as solvent to give isoxanthorin which was identified by direct comparison (TLC, UV and MS) with an authentic sample.

3-*O*-Benzoyl-6-*O*-methylaloesaponol I——A solution of 6-*O*-methylaloesaponol I (40 mg) dissolved in dimethylaniline (10 ml) and C_6H_6 (10 ml) was refluxed with C_6H_5COCl (7 ml) for 8 hr. The reaction mixture suspended in H_2O was extracted with hexane. The extract was repeatedly chromatographed over silica gel using hexane and C_6H_6 as solvents. The C_6H_6 eluate was recrystallized from ether to yield pale yellow needles of the benzoate (22 mg), mp 157—160°, MS m/e: 434.140 (M^+, Calcd. for $C_{25}H_{22}O_7$, 434.137); UV λ_{max}^{MeOH}

nm (log ε): 266 (4.3), 276 (4.4), 302 (3.9), 314 (4.0), 326 (3.9), 378 (4.0), 392 (4.0); IR ν_{max}^{KBr} cm^{-1}: 3440, 1730, 1720, 1630, 1610; NMR ($CDCl_3$) δ: 2.88 (3H, s, CH_3), 3.07, 3.20, 3.30, 3.42 (4H, dd, each, $J_{aa}=7$, $J_{ae}=4$, $J_{gem}=16$, C_2 and C_4–H), 3.92, 3.98 (3H, each, s, CH_3COO or CH_3O), 5.70 (1H, $W/2=16$, m, C_3–H), 6.83, 6.94 (1H, s, each, C_5 or C_{10}–H), 7.3—7.6 (3H, m, $C_{3'}$, $C_{4'}$, $C_{5'}$–H), 7.94 (2H, dd, $J=8$; 2, $C_{2'}$, $C_{6'}$–H), 14.94 (1H, s, OH).

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