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Studies on Monoterpene Glucosides. VII.[1,2] Iridoid Glucosides of *Paederia scandens*

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Four new glucosides, paederoside (II), paederosidic acid (III), scandoside (IV) and deacetylasperuloside (V) along with asperuloside (I) were isolated as iridoid glucosides from *Paederia scandens* (Lour.) Merrill var. *mairei* (Lévillé) Hara and their structures were established. II and III are sulfur-containing glucosides. III and V seem to be the artefacts formed during the extraction process.

The occurrence of asperuloside (I) and other iridoid glucosides in rubiaceous plants has often been reported.[2] It is known that *Paederia scandens* (Lour.) Merrill var. *mairei* (Lévillé) Hara, a herbaceous climbing plant, contains arbutin, oleanolic acid *etc.*[4] but there is no report on its iridoid glucosides. The present paper describes the isolation from it of four new iridoid glucosides and asperuloside (I) and elucidation of their structures.

The glucosides were isolated by the procedure shown in Chart 1. The fresh plants were extracted with hot water and the aqueous extract was treated with *n*-butanol using a continuous counter-current extraction method. After the treatment of both the aqueous layer (A–I) and the butanol extract (B–I) with lead acetate and hydrogen sulfide, the glucosides were purified by column chromatography on charcoal. On chromatography of the butanol extract (B–I), the fractions eluted with water–10% ethanol contained asperuloside (I) and those eluted with 20—30% ethanol contained paederoside (II). The 50% ethanol eluate was further fractionated by counter-current distribution (Fig. 1) to give paederosidic acid (III). After charcoal column chromatography of the water layer (A–I), the fractions eluted with water–10% ethanol were also subjected to counter-current distribution (Fig. 2 and 3) to give

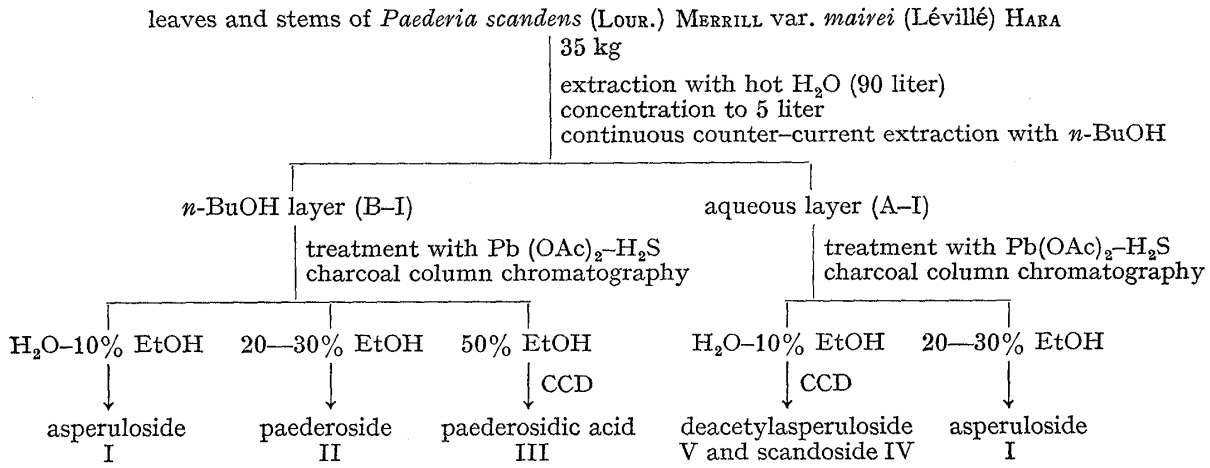


Chart 1

1) Part VI: H. Inouye and T. Arai, *Chem. Pharm. Bull.* (Tokyo), **16**, 1019 (1968).
2) A preliminary report of this work appeared in *Tetrahedron Letters*, **1968**, 683.
3) Location: *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*
4) T. Kurihara and N. Iino, *Yakugakuzasshi*, **84**, 479 (1964).

scandoside (IV) and deacetylasperuloside (V). The eluate with 20—30% ethanol contained asperuloside (I).

Although II, III, IV and V were new glucosides, III and V were not detected on a paper chromatogram of a freshly prepared extract of the plant, while III was not isolated from the methanol extract of the plant.[5] Therefore, III and V both seemed to be artefacts. Their structures will be mentioned later.

Asperuloside (I), $C_{18}H_{22}O_{11}\cdot\frac{1}{2}H_2O$, was isolated as colorless needles, mp 131—132°, $[\alpha]_D^{20}$ −193.7° (MeOH), which gave the acetate (VI) as colorless needles, mp 150—151°.

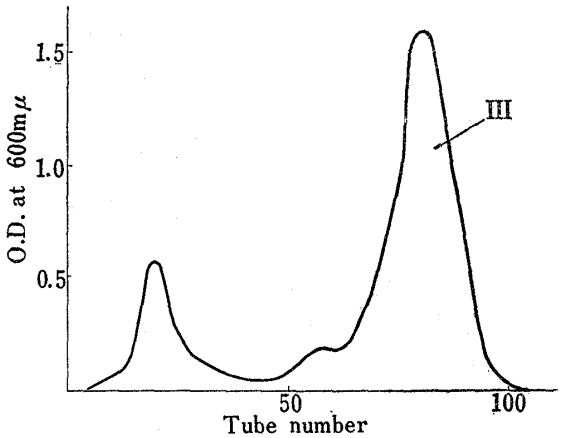


Fig. 1. Purification of Paederosidic Acid (III) by Counter-current Distribution

solvent system: *n*-BuOH–EtOH–H_2O (9:1:10)
transfers: 127 charge: 2 g
Assay was carried out as follows: 2.5 ml of 5 N H_2SO_4 were added to 0.5 ml of the lower layer and the solution was heated for 15 min at 80°. The O.D. of the resulting colored solution was determined at 600 mμ.

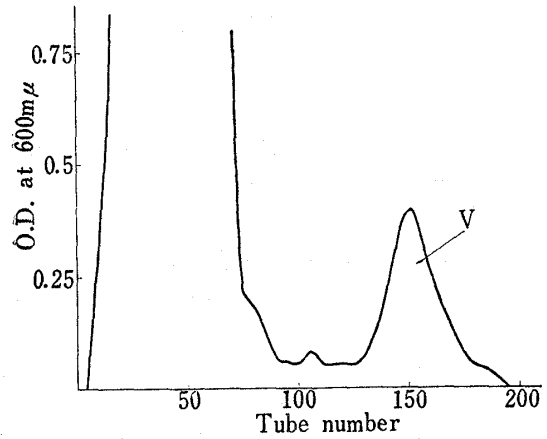


Fig. 2. Separation of Deacetylasperuloside (V) by Counter-current Distribution

solvent system: *n*-BuOH–EtOH–H_2O (10:1:10)
transfers: 900 charge: 4 g
Assaying procedure was the same as in Fig. 1.

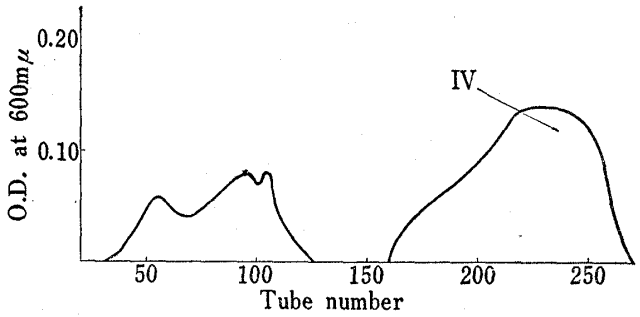


Fig. 3. Separation of Scandoside (IV) by Counter-current Distribution

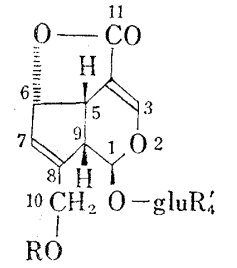
solvent system: *n*-BuOH–EtOH–H_2O (10:1:10)
transfers: 1118 charge: 4 g
Assaying procedure was the same as in Fig. 1.

The two compounds were identified with authentic samples of asperuloside from *Daphniphyllum macropodum* Miquel.[6] and its acetate (VI).

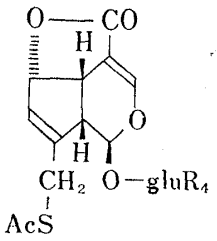
Paederoside (II), $C_{18}H_{22}O_{11}S\cdot 2H_2O$, was obtained as colorless needles, mp 122—123°, $[\alpha]_D^{24}$ −195.6° (MeOH). It showed an absorption maximum at 235 mμ (log ε 4.02) in the UV spectrum and bands at 1740, 1655 and 1610 cm^{-1} in the IR spectrum. Acetylation of II by the usual method yielded tetraacetate (VII), $C_{26}H_{30}O_{14}S\cdot H_2O$, mp 153.5—154°. Hydrolysis of II with β-glucosidase or dilute mineral acid resulted in a dark brown precipitate and an unpleasant odor like that of thioacetic acid. D-Glucose was also detected in the hydrolysate. Although the NMR spectrum of II closely resembles that of I, it differs in the following points. The signal for the acetyl group of I is at 7.88 τ, while that of II is at 7.65 τ. The signal of the C–10 protons appears at 5.24 τ in I and at 5.08 τ in II. Similar differences are found in the NMR spectra of the acetates VI and VII. For example, in VI, the signals for five acetyl

5) On extraction of the plant with MeOH, a substance with an NMR spectrum resembling that of paederosidic acid methyl ester was obtained. However, little material was available, and this compound has not yet been studied in detail. The possibility that III and V are artefacts is also supported by the observations reported in the next paper.

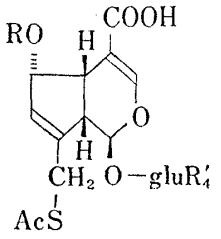
6) A.R. Trim, *Nature*, **167**, 485 (1951).



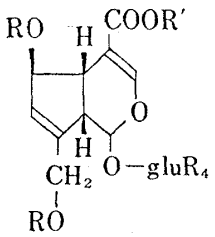
I : R=Ac, R'=H
V : R=H, R'=H
VI : R=Ac, R'=Ac



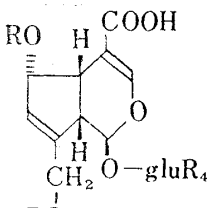
II : R=H
VII : R=Ac



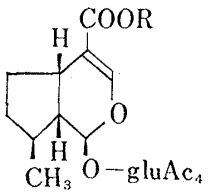
III : R=H, R'=H
X : R=Ac, R'=Ac



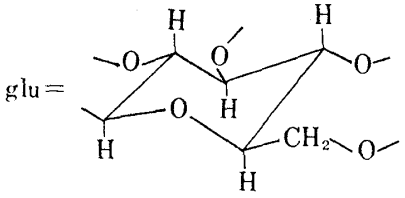
IV : R=H, R'=H
XI : R=Ac, R'=H
XII : R=H, R'=CH_3
XIII : R=Ac, R'=CH_3



VIII : R=H
IX : R=Ac



XIV : R=H
XV : R=CH_3



groups are at 7.89—8.00 τ, while in VII, the signals for four are found in the same region while the signal for a single acetyl group appears at 7.64 τ. Further, the signal for C–10 protons in VI appears at 5.34 τ, whereas in VII it is at 5.18 τ.

On hydrolysis of II with aqueous barium hydroxide, an acidic substance (VIII), $C_{16}H_{22}O_{11}\cdot H_2O$, mp 144—145°, was obtained along with thioacetic acid and acetic acid, which were detected by gas chromatography. The substance (VIII) was found to be identical with deacetylasperulosidic acid[7] obtained by hydrolysis of asperuloside (I). Previously, we reported the formation of asperuloside tetraacetate (VI) by acetylation of VIII.[7] In the present work we found that in the reaction oily deacetylasperulosidic acid hexaacetate (IZ) was also formed.

These data indicate that I and II have similar structures except that II has an S-acetyl group at C–10 instead of the acetoxyl group in the corresponding position in I.

Paederosidic acid (III), $C_{18}H_{24}O_{11}S\cdot 2H_2O$ $[\alpha]_D^{24}$ +28.2° (MeOH), is a strongly hygroscopic white powder. This compound showed a maximum at 233 mμ (log ε 3.98) in the UV spectrum and bands at 2750—2450, 1690 and 1635 cm^{-1} in the IR spectrum. The NMR spectrum of III, like II, showed a signal due to an acetyl group at 7.64 τ. Acetylation of III by the usual method furnished paederoside tetraacetate (VII) just as VI was formed from VIII. The fact that VII was formed from III suggests that II and III correspond to a lactone and the corresponding carboxylic acid. Finally, the possibility that the ester at the C–10 position of II and III was a thionic acid ester was eliminated on the basis of the IR spectrum of the triethylammonium salt of paederosidic acid (III). That is, the IR spectrum of this compound showed a carbonyl band at 1700 cm^{-1} besides the bands at 1540 and 1425 cm^{-1} attributed to a carboxylate, which indicates the presence of a thiol acetate. Accordingly, the structures of paederoside and paederosidic acid may be represented by formulae II and III. Although formation of VIII on hydrolysis of II is noteworthy, it could be explained by supposing that the S-acetyl group is located on an allylic carbon.

Scandoside (IV), $C_{16}H_{22}O_{11}\cdot 3/2H_2O$, mp 137—142°, $[\alpha]_D^{25}$ −53.3° (H_2O), is a hygroscopic colorless powder. Its NMR spectrum showed a doublet (J=1 cps) at 2.49 τ characteristic of a proton at the C–3 position of the iridoid glucoside, a multiplet at 4.15 τ attributable to a

7) H. Inouye, S. Ueda, M. Hirabayashi and N. Shimokawa, *Yakugaku Zasshi*, **86**, 943 (1966).

proton at the C–7 position, and a doublet (J=5 cps) at 4.65 τ due to a proton at the C–1 position. The NMR signals for the protons at C–5 and C–6′ overlapped each other around 6.55 τ and that for the proton at C–9 was a narrow multiplet at 6.89 τ. However, there were no clear triplets characteristic of the C–5 and C–9 protons of the series of deacetylasperulosidic acid (VIII) compounds. Scandoside (IV) itself does not have an acetyl group. Acetylation of IV by the usual method gave hexaacetate (XI), mp 156—159°, a white powder, as the sole product, unlike the result with VIII *etc.* Methylation of IV with diazomethane furnished scandoside methylester (XII), mp 105—109°, as a white powder. XI was also methylated with diazomethane yielding hexaacetate methylester (XIII), mp 132—134°, as fine colorless needles. XIII exhibited a carboxymethyl signal at 6.29 τ, a signal due to the C–10 protons at 5.27 τ and six acetyl signals around 7.95 τ in the NMR spectrum. Catalytic hydrogenation of hexaacetate (XI) over palladized charcoal yielded bisdesoxydihydrodeacetylasperulosidic acid tetraacetate (XIV), $C_{24}H_{32}O_{13}$, mp 183—186°, which was converted into the corresponding methylester (XV), mp 108°. The two compounds were identified with authentic specimens. The results revealed that scandoside (IV) has the same carbon skeleton as asperuloside (I) etc. From the foregoing data and the fact that hexaacetate (XI) is the sole product on acetylation of IV, the structure of scandoside (IV) was concluded to be 6-epideacetylasperulosidic acid.

Deacetylasperuloside (V), $C_{16}H_{20}O_{10}\cdot 2H_2O$, $[\alpha]_D^{20}$ −115.9° (MeOH), is a strongly hygroscopic white powder. The UV spectrum exhibited a maximum at 238 mμ (log ε 3.78), and its IR spectrum showed bands at 1735 and 1635 cm^{-1}. The NMR spectrum of this compound (V) is in general accord with that of asperuloside (I) except that the acetyl signal is absent and the signal due to the C–10 protons is higher than that of I at 5.24 τ. Acetylation of V afforded asperuloside tetraacetate (VI). On hydrolysis with aqueous barium hydroxide V gave deacetylasperulosidic acid (VIII). Therefore, it was concluded that the substance (V) must be the deacetylated asperuloside.

Among the five glucosides mentioned above, paederosidic acid (III) and deacetylasperuloside (V) seem to be artifacts as mentioned above while asperuloside (I), paederoside (II), and scandoside (III) are the intrinsic glucosides of the plant. The plant is called "Hekusokazura" in Japanese because of its unpleasant odor mainly due to thioacetic acid, which might be released from the bruised plant tissue through the hydrolysis of paederoside (II) with β-glucosidase followed by the degradation of the resulting aglucon. Compound (II) seems to be the first reported example of a naturally occurring sulphur-containing monoterpenoid. As the decarboxylated form of scandoside (III) is aucubin, the occurrence of this glucoside is very interesting.

Experimental[8]

Extraction and Isolation of the Glucosides——Thirty five kg of the leaves and stems of plants grown in Kyoto were collected in September and were immediately extracted three times with 30 liter portions of hot water. The extract was concentrated *in vacuo* to *ca.* 5 liter using a thin-layer evaporator. The aqueous extract was then extracted with *ca.* 5 liter of *n*-BuOH using a continuous counter-current extraction apparatus to give an aqueous layer (A–I) and a BuOH-layer (B–I). Each layer was concentrated to dryness *in vacuo* and dissolved in MeOH. After treatment with lead acetate and H_2S, the filtrates were

8) NMR spectra were determined on a Varian A–60 spectrometer in D_2O with DSS and in other solvents with TMS as internal standard. All melting points are given as uncorrected values. Ascending paper chromatography was carried out on Toyo Roshi No. 50 filter paper with the upper layer of an *n*-BuOH–AcOH–H_2O (4:1:5 v/v) mixture. Silica gel G acc. to Stahl (Merck) was used for thin-layer chromatography. A solution of 2-aminobiphenyl hydrogen oxalate in acetone was used for detection of glucose and dil. HCl for iridoid glucosides. Spots were detected after spraying by ironing the paper. The former reagent gave a brown color with glucose and the latter a blue color with glucosides. Silica gel (Mallinckrodt) was used for column chromatography. Counter-current distribution was performed in a Mitamura automatic all-glass apparatus with 300 tubes.

concentrated and again dissolved in H_2O. The aqueous solutions were chromatographed on charcoal with H_2O–EtOH mixtures as solvent. The eluates were monitored by paper chromatography and appropriate fractions were combined for further treatments. For column chromatography of the BuOH-layer (B–I), 80 g of charcoal and 80 g of celite were used for preparation of the column, and for the aqueous layer (A–I), 200 g each of charcoal and celite.

Asperuloside (I)——The BuOH–layer (B–I) was chromatographed over charcoal. The fractions eluted with H_2O–10% EtOH yielded asperuloside (I) as almost the sole iridoid component. The eluate was concentrated *in vacuo* and the residue was recrystallized from H_2O to give 1.8 g of colorless needles, mp 131—132°, $[\alpha]_D^{26}$ −193.7° (c=0.54, MeOH), UV λ_{max}^{MeOH} mμ (log ε) 235.5 (3.80). *Anal.* Calcd. for $C_{18}H_{22}O_{11}\cdot\frac{1}{2}H_2O$: C, 51.07; H, 5.48. Found: C, 56.79; H, 5.51. This was acetylated by the usual method with pyridine and Ac_2O to give tetraacetate (VI) as colorless needles, mp 150—151°, $[\alpha]_D^{28}$ −164.6° (c=1.05, MeOH). Both I and VI were identical with authentic samples. Asperuloside (I) (3 g) was also obtained when the aqueous layer (A–I) was chromatographed over charcoal with 20—30% EtOH as eluent.

Paederoside (II)——On chromatography of the BuOH-layer (B–I) on charcoal, fractions eluted with 20—30% EtOH were combined and concentrated *in vacuo*. The residue was recrystallized from H_2O to give colorless needles of II, mp 122—123°. Yield 15 g. $[\alpha]_D^{24}$ −195.6° (c=0.41, MeOH), UV λ_{max}^{MeOH} mμ (log ε) 235 (4.02), IR (KBr) cm^{-1}: 1740, 1655, 1610. *Anal.* Calcd. for $C_{18}H_{22}O_{10}S\cdot 2H_2O$: C, 46.34; H, 5.62; S, 6.88. Found: C, 46.42; H, 5.45; S, 7.04.

Paederosidic Acid (III)——After chromatography of the BuOH-layer (B–I) on charcoal, fractions eluted with 50% EtOH were concentrated *in vacuo* to give 11 g of residue, 2 g of which were fractionated by counter-current distribution to give the distribution curve shown in Fig. 1. The fractions in tubes 65—95 were combined and redistributed in the same solvent system. A similar distribution curve was again obtained. The fractions corresponding to each band in the distribution curve were combined and the solvent was removed *in vacuo*. A methanolic solution of the residue was chromatographed on a charcoal column (1×2 cm, prepared using the same relative amounts of charcoal and celite). After evaporation of the eluate *in vacuo*, the residue was taken up in H_2O and lyophilized to give 0.6 g of paederosidic acid (III) as a white, very hygroscopic powder. mp 127—130°, $[\alpha]_D^{24}$ +28.2° (c=0.51, MeOH), UV λ_{max}^{MeOH} mμ (log ε): 233 (3.98), IR (KBr) cm^{-1}: 2700—2450, 1690, 1640. *Anal.* Calcd. for $C_{18}H_{24}O_{11}S\cdot 2H_2O$: C, 44.62; H, 5.83. Found: C, 44.15; H, 5.78. The other fractions obtained by counter-current distribution have not yet been investigated.

Deacetylasperuloside (V)——D-Glucose was detected by PPC of the aqueous eluate obtained by charcoal column chromatography of the aqueous layer (A–I). The eluate with 10% EtOH contained four iridoids with *Rf* values of 0.05—0.35 on paper chromatography. These fractions were combined and evaporated *in vacuo*. After passing an aqueous solution of the residue through a charcoal column (1×2 cm), the eluate was lyophilized to afford *ca.* 20 g of residue. One fifth of the residue was fractionated by counter-current distribution. The distribution curve is shown in Fig. 2. The fractions in tubes 129—196 were combined and the solvent was removed *in vacuo*. The residue was dissolved in a small amount of MeOH and the solution was passed through a charcoal column (1×2.5 cm) with MeOH as eluent. The eluate was evaporated to dryness *in vacuo*. The residue was dissolved in H_2O and lyophilized to give deacetylasperuloside (V) as a white powder. Yield 110 mg, mp 113—118°, $[\alpha]_D^{20}$ −115.9° (c=1.57, MeOH), UV λ_{max}^{EtOH} mμ (log ε): 238 (3.78), IR (KBr) cm^{-1}: 1735, 1655. *Anal.* Calcd. for $C_{16}H_{20}O_{10}\cdot 2H_2O$: C, 47.06; H, 5.92. Found: C, 47.40; H, 6.50.

Scandoside (IV)——The fractions in tubes 11—70 of the preceding counter-current fractionation were combined and evaporated *in vacuo* to furnish 4.12 g of residue containing a small amount of solvent. This was again subjected to counter-current fractionation to give the distribution curve shown in Fig. 3. The fractions in tubes 201—270 were combined and evaporated to dryness *in vacuo*. The residue was passed through a charcoal column (1×3 cm) with MeOH as eluent. The eluate was evaporated *in vacuo* and dissolved in H_2O. The aqueous solution was lyophilized to give scandoside (IV) as a white powder. Yield 1.8 g, mp 137—142°, $[\alpha]_D^{25}$ −53.3° (c=0.51, H_2O). UV λ_{max}^{EtOH} mμ (log ε): 235 (4.16), IR (KBr) cm^{-1}: 2750—2400, 1680, 1635. *Anal.* Calcd. for $C_{16}H_{22}O_{11}\cdot 3/2H_2O$: C, 46.04; H, 6.04. Found: C, 46.13; H, 5.77. The fractions in tubes 31—70 and 71—130 have not yet been examined.

Acetylation of Paederoside (II)——II was acetylated by the usual method with pyridine and Ac_2O to furnish paederoside tetraacetate (VII) as colorless needles, mp 153.5—154°, $[\alpha]_D^{27}$ −143.1° (c=0.39, MeOH). IR (KBr) cm^{-1}: 1765, 1710, 1665. NMR ($CDCl_3$) τ: 2.80 (1H, d, J=2 cps, C-3), 4.20 (1H, m, C-7), 4.29 (1H, d, J=2 cps, C-1), 5.20 (2H, m, C-10), 7.64 (s, CH_3CO-S-), 7.85—8.00 (4×CH_3COO–). *Anal.* Cacld. for $C_{26}H_{30}O_{14}S\cdot H_2O$: C, 50.64; H, 5.24; S, 5.20. Found: C, 50.28; H, 5.04; S, 5.26.

Hydrolysis of Paederoside (II) with Mineral Acid or Emulsin——i) A solution of 10.4 mg of paederoside (II) in 2 ml of 0.5% HCl was heated under reflux. The solution became dark blue and then a dirty green precipitate formed. The solution was neutralized with a small amount of Amberlite IR–4B and the filtrate was evaporated *in vacuo*. The residue was subjected to paper chromatography and identified with D-glucose.

ii) To a solution of 15 mg of paederoside (II) in 1.5 ml of acetate buffer solution (pH 4.9) was added 0.5 ml of an aqueous solution of emulsin (1%). The reaction mixture was allowed to stand at 37° for 24 hr. A brown precipitate formed after a few hours and at the same time a stench characteristic of thioacetic acid was noted.

Hydrolysis of Paederoside (II) with $Ba(OH)_2$——i) Formation of Deacetylasperulosidic Acid (VIII): To a solution of 1 g of II in *ca.* 10 ml of H_2O were added 2.5 g of $Ba(OH)_2$ to adjust the pH to 13. The mixture was stood at room temperature for 17 hr, the pH was adjusted to 3 with Amberlite IR 120 and the mixture was filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was recrystallized from EtOH to give 200 mg of VIII as colorless needles, mp 144—145°, $[\alpha]_D^{31}$ +32.8° (c=1.86, MeOH), UV λ_{max}^{MeOH} mμ (log ε): 235 (4.05). IR (KBr) cm^{-1}: 2700—2400, 1680, 1625. *Anal.* Calcd. for $C_{16}H_{22}O_{11}\cdot H_2O$: C, 47.06; H, 5.92. Found: C, 47.10; H, 6.22.

ii) Detection of Thioacetic Acid: To a solution of 0.3 g of paederoside (II) in *ca.* 10 ml of H_2O was added 0.6 g of $Ba(OH)_2$. After standing the mixture at room temperature for 15 hr, 10% H_2SO_4 was added to adjust the pH to 2. The solution was extracted with ether and the ether layer was dried over anhydrous Na_2SO_4. The solvent was removed at low temperature, to give a small amount of yellowish oil, which was subjected to gas chromatographic analysis and the presence of thioacetic acid and acetic acid was confirmed. A Shimadzu gas chromatograph GC-1B, equipped with a flame ionization detector, was used for this purpose. Column: 1,4 BDS on chromosorb W 0.3×300 cm. The column temperature was set to 110° and N_2 was passed through the column at a flow rate 80 ml/min. Flow rate of H_2: 60 ml/min. Retention time: Thioacetic acid 1.7 min, acetic acid 5.9 min.

Acetylation of Deacetylasperulosidic Acid (VIII)——A solution of 100 mg of VIII in 2 ml each of pyridine and Ac_2O was allowed to stand overnight. Ac_2O was decomposed by pouring the reaction mixture into ice water and the solvent was removed *in vacuo*. The residue was recrystallized first from EtOH and then from MeOH to give asperuloside tetraacetate (VI) as colorless needles, mp 153°. Yield 40 mg. After recrystallization from EtOH the mother liquor was evaporated to dryness. The residue was chromatographed on a silica gel column (10 g) and eluted with increasing concentration of MeOH in $CHCl_3$, using in succession, $CHCl_3$, $CHCl_3$–MeOH (99:1 v/v), $CHCl_3$–MeOH (98: 2v/v) and finally $CHCl_3$–MeOH (97: 3 v/v), which eluted 30 mg of deacetylasperulosidic acid hexaacetate (IX), a colorless oil. IR ($CHCl_3$) cm^{-1}: 1740, 1685, 1640. NMR ($CDCl_3$) τ: 2.34 (1H, d, J=1 cps, C-3), 6.75 (1H, t, C-5), 7.30 (1H, t, C-9), 7.85—8.06 (4×CH_3COO). *Anal.* Calcd. for $C_{28}H_{34}O_{17}\cdot 1/2H_2O$: C, 51.62; H, 5.42. Found: 51.48; C, H, 5.69.

Acetylation of Paederosidic Acid (III)——To a solution of 60 mg of paederosidic acid (III) in 0.6 ml of pyridine was added 0.6 ml of Ac_2O and the mixture was allowed to stand overnight and then poured into ice water. The resulting syrup was dissolved in $CHCl_3$ and the solution was passsed through a silica gel column (15 g) eluting with the same solvent. The first eluate was evaporated and the residue was recrystallized. The first eluate was evaporated and the residue was recrystallized from EtOH to give paederoside tetraacetate (VII) as colorless plates, mp 156.5—158°. Yield 12 mg.

Triethylammonium Salt of Paederosidic Acid (III)——To a solution of 15 mg of paederosidic acid (III) in 3 ml of H_2O was added *ca.* 0.15 ml of Et_3N with stirring and the solution was lyophilized. The resulting powder was subjected to IR analysis. IR (KBr) cm^{-1}: 1690, 1540, 1425. Then the triethylammonium salt of III obtained was dissolved in 5 ml of H_2O and passed through a column of 5 ml of Amberlite IR–120 (H–from). The eluate was evaporated *in vacuo* and then lyophilized, yielding 13 mg of a white powder, which had identical IR and NMR spectra to III.

Acetylation of Scandoside (IV)——IV (150 mg) was acetylated by the usual method. The resulting acetate was dissolved in *t*-BuOH and lyophilized to give scandoside hexaacetate (XI) as a white powder, mp 156—159°, $[\alpha]_D^{24}$ −97.4° (c=1.21 ,MeOH). Yield 174 mg. UV λ_{max}^{EtOH} mμ (log ε): 230 (4.01), IR (KBr) cm^{-1}: 1755—1710, 1640, NMR ($CDCl_3$) τ: 2.51 (1H, s, C-3), 7.89—8.03 (6×CH_3COO). *Anal.* Cacld. for $C_{28}H_{34}O_{17}\cdot 1/2H_2O$: C, 51.46; H, 5.76. Found: C, 51.83; H, 5.65.

Methylation of Scandoside (IV)——To a solution of 50 mg of IV in 5 ml of MeOH a small excess of CH_2N_2–ether was added. The solvent was evaporated *in vacuo* and the aqueous solution of the residue was lyophilized to give XII as a white powder, mp 105—109°. Yield 50 mg. $[\alpha]_D^{22}$ −33.7° (c=1.33, MeOH). UV λ_{max}^{EtOH} mμ (log ε): 239 (4.04). IR (KBr) cm^{-1}: 1695, 1635. NMR (D_2O) τ: 2.50 (1H, d, J=1 cps, C-3), 4.62 (1H, d, J=5 cps, C-1), 6.25 (CH_3COS–). *Anal.* Calcd. for $C_{17}H_{24}O_{11}\cdot 3/2H_2O$: C, 47.32; H, 6.31. Found: C, 47.68; H, 6.33.

Methylation of Scandoside Hexaacetate (XI)——To an ethereal solution of 50 mg of XI was added CH_2N_2–ether. After standing for a while the reaction mixture was evaporated *in vacuo*. Recrystallization of the residue from ether afforded scandoside hexaacetate methyl ester (XIII) as fine colorless needles. Yield 40 mg. mp 132—134°, $[\alpha]_D^{20}$ −85.4° (c=0.85, MeOH). UV λ_{max}^{EtOH} mμ (log ε): 233 (4.00). IR (KBr) cm^{-1}: 1740, 1710, 1640. NMR ($CDCl_3$) τ: 2.52 (1H, s, C-3), 4.10 (1H, on account of the overlapping with other signals, the detailed type was not ascertained. C-1), 5.27 (2H, m, C-10), 6.29 (3H, s, $-COOCH_3$), 6.75 (2H, m, C-5, C-9), 7.87—8.07 (6×CH_3COO). *Anal.* Cacld. for $C_{29}H_{36}O_{17}$: C, 53.05; H, 5.52. Found: C, 52.96; H, 5.36.

Catalytic Hydrogenation of Scandoside Hexaacetate (XI)——A solution of 50 mg of XI in 20 ml of AcOH was shaken with H_2 in the presence of a catalyst prepared by the usual method from 50 mg of activated charcoal (Norite) and 0.3 ml of 5% $PdCl_2$ solution. After the uptake of hydrogen ceased, the catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was recrystallized first from aqueous EtOH and then from MeOH to give XIV as colorless needles, mp 183—186°. Yield 10 mg. This was identified with an authentic sample by measuring the mixed melting point and IR spectrum. IR (KBr) cm^{-1}: 1755,

1720, 1680, 1645. *Anal.* Calcd. for $C_{24}H_{32}O_{13}$: C, 54.55; H, 6.11. Found: C, 54.41; H, 6.18. XIV was methylated by the usual method with CH_2N_2–ether to afford methyl ester (XV) as colorless neeldles, mp 108°. Its identity with an authentic sample was demonstrated by measuring the mixed melting point and comparison of the IR spectra. IR (KBr) cm^{-1}: 1750, 1710, 1640. *Anal.* Calcd. for $C_{25}H_{34}O_{13}$: C, 55.34; H, 6.32. Found: C, 55.29; H, 6.43.

Acetylation of Deacetylasperuloside (V)——A solution of 50 mg of V in 1 ml each of pyridine and Ac_2O was stood overnight and then poured into ice water. Recrystallization of the resulting precipitate afforded 15 mg of colorless needles, mp 150—152°, which were shown to be identical with asperuloside tetraacetate (VI) by measuring the melting point and comparison of IR and NMR spectra. *Anal.* Calcd. for $C_{26}H_{30}O_{15}$: C, 53.60; H, 5.20. Found: C, 53.54; H, 5.01.

Hydrolysis of Deacetylasperuloside (V)——To a solution of 70 mg of V in 3 ml of H_2O was added a saturated aqueous solution of $Ba(OH)_2$ until the pH of the solution became 13. The solution was stood overnight, adjusted to pH 3 with Amberlite IR 120 and filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in MeOH, treated with charcoal (by column chromatography), and the solvent was again evaporated off. Recrystallization of the residue from EtOH afforded VIII as colorless needles, mp 145—147°. Yield 30 mg. This product had the same melting point and IR spectrum as an authentic specimen.

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