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Isolation and Structure of Thalictoside

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Thalictoside (**1**), a novel glycoside containing a nitro group, was isolated from *Thalictrum aquilegifolium* and the structure was shown to be 4-hydroxy-1-(2-nitroethyl)benzene 4-*O*-β-D-glucopyranoside on the basis of chemical transformations, spectral analysis and synthesis. Several known compounds were also isolated.

Keywords——Ranunculaceae; *Thalictrum aquilegifolium*; thalictoside; phenolic glycoside; *O*-methylthalicberine; ^{13}C-NMR

Thalictrum aquilegifolium (Ranunculaceae) is a plant that is poisonous to domestic animals.[1] In the course of our research on the poisonous substances of this plant, we have isolated a novel glycoside containing a nitro group, named thalictoside (**1**). A part of this work has been reported in a preliminary communication.[2] The present paper mainly gives a full account of the isolation and structure elucidation of **1**.

Extraction and separation were carried out according to the procedure shown in Chart 1. From fraction A, hydrocarbons, alcohols, β-sitosterol, oleanolic acid, maslinic acid and β-sitosteryl β-D-glucoside were obtained. A benzylisoquinoline alkaloid, *O*-methylthalicberine was isolated from fraction C and sugars were obtained from fraction D.

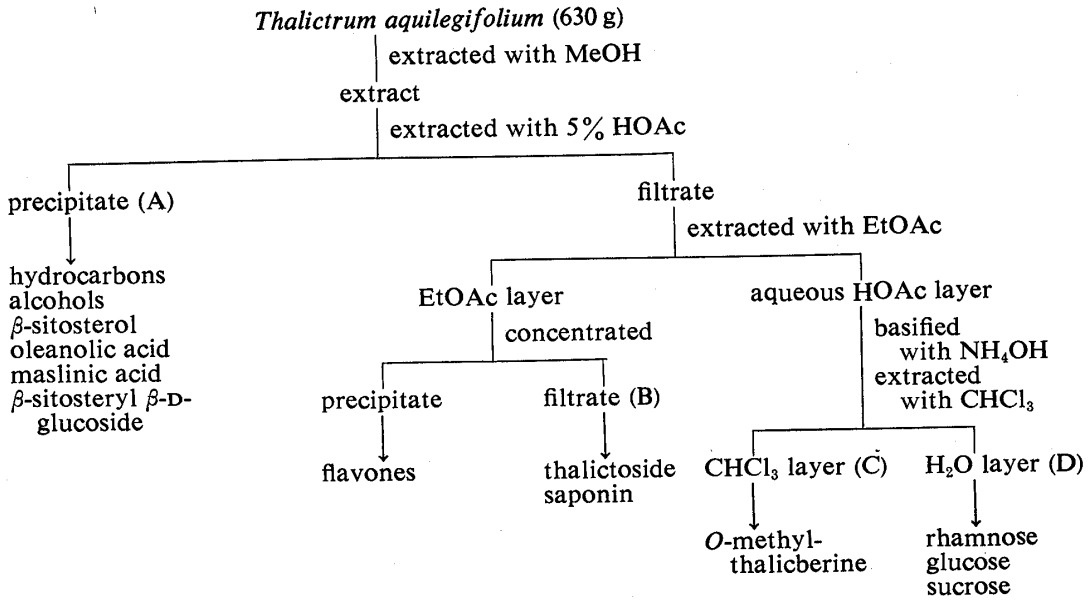

Chart 1

Fraction B was chromatographed on a silica gel column and eluted with benzene–ethyl acetate (1 : 10) to give colorless crystals of **1**, mp 102—103 °C, $C_{14}H_{19}NO_8$. The infrared (IR) spectrum of **1** suggested the presence of hydroxy groups (3350 cm^{-1}), an aromatic ring

(1610 cm^{-1}) and a nitro group (1550 and 1380 cm^{-1}). Absorption maxima at 270 and 218 nm of the ultraviolet (UV) spectrum also supported the presence of a nitro group.

Acetylation of **1** with acetic anhydride–pyridine gave a tetraacetate **3** as colorless crystals, mp 167—168 °C, $C_{22}H_{27}NO_{12}$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed the presence of four acetoxy groups [δ 2.06, 2.08 and 2.10 (12H, each s)], a *para*-substituted benzene ring [δ 6.94 and 7.14 (each 2H, d, J=8 Hz)] and a $-CH_2CH_2-$ group [δ 3.28 and 4.58 (each 2H, t, J=7 Hz)]. Acid hydrolysis of **1** with MeOH–10% HCl gave D-(+)-glucose and an aglycone **2** as a pale yellow oil, which gave a red-purple color with 2% aqueous ferric chloride. The high-resolution mass spectrum (MS) of **2** indicated a molecular formula of $C_8H_9NO_3$ and the IR spectrum still suggested the presence of a nitro group (1550 and 1380 cm^{-1}). The ^{1}H-NMR spectrum of **1** exhibited a signal of one anomeric proton at δ 5.45 (1H, d, J=7 Hz), which indicated the presence of a β-glucopyranoside linkage.

These data led to the assignment of thalictoside as 4-hydroxy-1-(2-nitroethyl)benzene 4-*O*-β-D-glucopyranoside (**1**). Furthermore, this structure **1** is well supported by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) data.

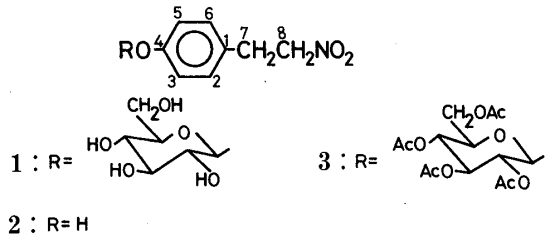

Chart 2

Thalictoside (**1**) was synthesized as follows. *p*-Hydroxybenzaldehyde was condensed with nitromethane in acetic acid in the presence of ammonium acetate to yield nitrostyrene (**4**). The reduction product of **4** with sodium borohydride in ethanol was identical with the aglycone **2** in terms of the MS, IR and ^{1}H-NMR spectra. This phenolic aglycone **2** was condensed with 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (**5**) in toluene in the presence of cadmium carbonate.[3] Two isomeric products were separated by preparative thin layer chromatography (TLC) on silica gel. These isomers (β- and α-anomers) were clearly distinguished by ^{1}H-NMR data for the sugar moieties.[4] Furthermore, the β-anomer was identical with **3** in terms of the IR and ^{1}H-NMR spectra, specific rotation and mixed melting point determination. Treatment of the β-anomer **3** and α-anomer **6** with 5% KOH in methanol gave the β-anomeric glycoside and α-anomeric glycoside, respectively. The β-anomeric glycoside was identical with the naturally occurring glycoside **1** on the basis of IR and ^{1}H-NMR spectral and specific rotation comparisons and mixed melting point determination. The ^{1}H-NMR spectrum of the α-anomeric glycoside **7** showed one anomeric proton at δ 5.88 (1H, d, J=3 Hz) for the α-anomer.

Thalictoside (**1**) is an unusual natural product which is a new addition to the small number of naturally occurring aliphatic nitro compounds, *e.g.* miserotoxin[5] and endecaphyllins.[6]

A toxicity test, performed by injection of **1** into the abdominal cavities of mice, did not show the expected toxicity.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The following instruments were used: optical rotation, Jasco Dip-4 polarimeter; UV spectra, Hitachi 124 spectrophotometer; IR spectra, Hitachi 215 spectrometer; optical rotatory dispersion (ORD), Jasco J-20; ^{1}H-NMR and ^{13}C-NMR

spectra, JEOL PS-100 spectrometer (100 MHz) and JEOL FX-100 spectrometer (25 MHz) with tetramethylsilane ($\delta=0$) as an internal reference; MS, Hitachi RMU-7L. The abbreviation used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Column chromatography was carried out on silica gel (Merck 60, 70—230 mesh ASTM). Silica gel 60 F_{254} (Merck plates) was used for TLC and preparative TLC. The spots were detected by spraying the plates with 2% aqueous $FeCl_3$, exposing them to I_2 or heating them after spraying with $Ce(SO_4)_2$–10% H_2SO_4 reagent.

Extraction and Isolation——Dried whole plant (including the roots, 630 g) of *Thalictum aquilegifolium* collected in 1977 in Shizuoka Prefecture, Japan, was extracted with hot MeOH and the MeOH solution was evaporated to 300 ml under reduced pressure. The MeOH extract was extracted with 300 ml of 5% HOAc and the precipitate A was filtered off. The aqueous HOAc solution was extracted with EtOAc and the precipitate was filtered off from the concentrated EtOAc extract B. The aqueous HOAc layer, after extraction with EtOAc, was basified with NH_4OH and extracted with $CHCl_3$ to give the chloroform layer C and the water layer D.

Fraction A was separated on a silica gel column, eluting with hexane, hexane–C_6H_6, C_6H_6 and C_6H_6–EtOAc, successively. The hexane eluate afforded hydrocarbons. The hexane–C_6H_6 (1 : 1) eluate yielded alcohols, the C_6H_6 eluate gave β-sitosterol, the C_6H_6–EtOAc (10 : 1) eluate gave oleanolic acid, the C_6H_6–EtOAc (1 : 1) eluate gave maslinic acid, and the C_6H_6–EtOAc (1 : 5) eluate gave β-sitosteryl β-D-glucoside. Fraction B was subjected to silica gel column chromatography and the C_6H_6–EtOAc (1 : 10) eluate gave **1**. From the $CHCl_3$–MeOH (50 : 1) eluate of silica gel column chromatography of fraction C, *O*-methylthalicberine was isolated. From the H_2O eluate of charcoal column chromatography of fraction D, sugars were obtained.

Hydrocarbons——IR ν_{max}^{KBr} cm^{-1}: 730, 720. Gas liquid chromatography (GLC) operating conditions were as follows. Column: 10% SE-30 on Chromosorb W (AW), temp.: 200→300 °C (program rate, 2 °C/min). The relative quantities determined from the results of gas liquid chromatography-mass spectroscopy (GC-MS) were as follows. *n*-$C_{23}H_{48}$, 0.8%; *n*-$C_{24}H_{50}$, 0.3%; *n*-$C_{25}H_{52}$, 3.5%; *n*-$C_{26}H_{54}$, 0.6%; *n*-$C_{27}H_{56}$, 6.8%; *n*-$C_{28}H_{58}$, 2.9%; *n*-$C_{29}H_{60}$, 43.4%; *n*-$C_{30}H_{62}$, 5.6%; *n*-$C_{31}H_{64}$, 28.7%; *n*-$C_{32}H_{66}$, 0.5%; *n*-$C_{33}H_{68}$, 4.8%; *n*-$C_{34}H_{70}$, –; *n*-$C_{35}H_{72}$, 0.7%.

Alcohols——IR ν_{max}^{KBr} cm^{-1}: 3500—3300, 730, 720. The sample was acetylated and subjected to GC-MS. The GLC operating conditions were the same as in the case of hydrocarbons. Relative quantities were as follows. *n*-$C_{28}H_{57}OAc$, 2.0%; *n*-$C_{29}H_{59}OAc$, 0.7%; *n*-$C_{30}H_{61}OAc$, 12.5%; *n*-$C_{31}H_{63}OAc$, 5.6%; *n*-$C_{32}H_{65}OAc$, 21.0%; *n*-$C_{33}H_{67}OAc$, 4.5%; *n*-$C_{34}H_{69}OAc$, 22.1%; *n*-$C_{35}H_{71}OAc$, 0.3%; *n*-$C_{36}H_{73}OAc$, 1.9%.

β-Sitosterol——mp 140—141 °C (MeOH). Identical with an authentic sample.

Oleanolic Acid——Purified as the acetate, mp 256—258 °C (MeOH). Identical with an authentic sample.

Maslinic Acid——Purified as the diacetate, mp 231—233 °C (hexane). Identical with an authentic sample.

β-Sitosteryl β-D-Glucoside——Purified as the tetraacetate, mp 168—169 °C (MeOH). Identical with an authentic sample.

Thalictoside (1)——Purified by preparative TLC using $CHCl_3$–MeOH (7 : 3) and recrystallized from MeOH–$CHCl_3$, colorless crystals 62 mg, mp 102—103 °C, $[\alpha]_D^{25}$ −48.8° ($c=1.1$, MeOH). *Anal.* Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 50.81; H, 5.73; N, 4.13. IR ν_{max}^{KBr} cm^{-1}: 3350, 1610, 1550, 1380. UV $\lambda_{max}^{H_2O}$ nm (ε): 218 (7532), 270 (1063). ^{1}H-NMR (in C_5D_5N) δ: 5.45 (1H, d, $J=7$ Hz, $C_{1'}$-H). ^{13}C-NMR (in C_5D_5N) δ: 157.6 (s, C-4), 129.9 (s and d, C-1, 2, 6), 117.0 (d, C-3, 5), 102.0 (d, C-1′), 78.6 (d, C-3′ or 5′), 78.2 (d, C-5′ or 3′), 76.6 (t, C-8), 74.7 (d, C-2′), 71.1 (d, C-4′), 62.2 (t, C-6′), 32.6 (t, C-7).

Acid Hydrolysis of 1——A solution of **1** (30 mg) in MeOH (6 ml) and 10% HCl (2 ml) was refluxed for 2 h, and the reaction mixture was extracted with EtOAc. The EtOAc extract was purified by preparative TLC using C_6H_6–EtOAc (4 : 1) to give **2** (11 mg) as a pale yellow oil which gave a red-purple color with 2% $FeCl_3$. TLC, *Rf* (solvent): 0.51 (C_6H_6 : EtOAc = 3 : 1), 0.44 (C_6H_6 : MeOH = 10 : 1), 0.33 (hexane : EtOAc = 2 : 1). In all solvent systems, only one spot could be detected. High-resolution MS *m/z*: M^+ Calcd for $C_8H_9NO_3$, 167.0583. Found: 167.0576. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3300, 1610, 1550, 1380. ^{1}H-NMR (in $CDCl_3$) δ: 3.20, 4.54 (each 2H, t, $J=8$ Hz, C_7-H, C_8-H), 6.74, 7.20 (each 2H, d, $J=8$ Hz, $C_{3,5}$-H, $C_{2,6}$-H). The aqueous layer of the hydrolysate was treated with Amberlite CG-400 and evaporated *in vacuo* to give the sugar moiety. The sugar was identified as glucose by GLC comparison of the trimethylsilylated sugar with an authentic sample. The GLC operating conditions were as follows. Column: 5% OV-1 on Chromosorb W (AW), temp.: 175 °C. Furthermore, the sugar showed a plain curve identical with that of the authentic D-(+)-glucose in the ORD spectrum.

Thalictoside Tetraacetate (3)——Acetylation of **1** (15 mg) with acetic anhydride–pyridine in the usual way gave **3** (19 mg) as colorless crystals, recrystallized from MeOH, mp 167—168 °C, $[\alpha]_D^{21}$ −2.6° ($c=1.3$, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{27}NO_{12}$: C, 53.11; H, 5.47; N, 2.81. Found: C, 52.91; H, 5.29; N, 2.55. IR ν_{max}^{KBr} cm^{-1}: 1745, 1550, 1370, 1230. ^{1}H-NMR (in $CDCl_3$) δ: 2.06 (3H, s, $OCOCH_3$), 2.08 (6H, s, $2\times OCOCH_3$), 2.10 (3H, s, $OCOCH_3$), 3.28, 4.58 (each 2H, t, $J=7$ Hz, C_7-H, C_8-H), 3.90 (1H, br m, $C_{5'}$-H), 4.20 (2H, m, $C_{6'}$-H), 4.96—5.34 (4H, m, $C_{1',2',3',4'}$-H), 6.94, 7.14 (each 2H, d, $J=8$ Hz, $C_{3,5}$-H, $C_{2,6}$-H).

***p*-Hydroxy-β-nitrostyrene (4)**——The procedure of Iida *et al.*[7] was used to prepare **4**. Recrystallization from H_2O gave pale yellow crystals, mp 158—162 °C (lit.[8] 154—160 °C). *Anal.* Calcd for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 57.98; H, 4.35; N, 8.29.

Reduction of 4 with Sodium Borohydride——$NaBH_4$ (3.4 g) was added to a solution of **4** (5 g) in EtOH (400 ml)

and the mixture was stirred at room temp. for 24 h. The reaction mixture was acidified with 10% HCl and evaporated. The concentrated reaction mixture was extracted with EtOAc. The EtOAc extract was purified by a silica gel column using hexane–C_6H_6 (5 : 1) to give a pale yellow oil (2.8 g), which was identified as **2** from the MS, IR and ^{1}H-NMR spectra.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucosyl Bromide (5)——mp 88—89 °C (lit.[9] 87—88 °C).

Condensation of 2 with 5——A solution of **2** (334 mg) and **5** (820 mg) in toluene (60 ml) was refluxed for 24 h in the presence of cadmium carbonate (344 mg). The precipitate was filtered off and washed with $CHCl_3$. The filtrate and the washings were combined, washed with 5% KOH and worked up in the usual way to give the crude mixture (255 mg). A portion of this mixture (150 mg) was purified by preparative TLC using C_6H_6–EtOAc (4 : 1) to give the β-anomer (84 mg) and the α-anomer (43 mg). The β-anomer was recrystallized from MeOH to give colorless crystals which were identical with **3** on the basis of IR and ^{1}H-NMR spectral and specific rotation comparisons and mixed melting point determination. Recrystallization of the α-anomer **6** from EtOH gave colorless needles, mp 111—112 °C, $[\alpha]_D^{21}$ +147.2° (c=1.4, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{27}NO_{12}$: C, 53.11; H, 5.47; N, 2.81. Found: C, 53.16; H, 5.35; N, 2.95. IR ν_{max}^{KBr} cm^{-1}: 1742, 1730, 1555, 1380, 1230. ^{1}H-NMR (in $CDCl_3$) δ: 2.02 (3H, s, $OCOCH_3$), 2.04 (3H, s, $OCOCH_3$), 2.06 (6H, s, 2 × $OCOCH_3$), 3.24, 4.56 (each 2H, t, J=7 Hz, C_7-H, C_8-H), 4.08 (1H, br m, $C_{5'}$-H), 4.16 (2H, m, $C_{6'}$-H), 4.90—5.24 (2H, m, $C_{2',4'}$-H), 5.66 (1H, t, J=9 Hz, $C_{3'}$-H), 5.68 (1H, d, J=3 Hz, $C_{1'}$-H), 6.98, 7.12 (each 2H, d, J=8 Hz, $C_{3,5}$-H, $C_{2,6}$-H).

Alkaline Hydrolysis of 3——A solution of **3** (53 mg) in MeOH (5 ml) and 5% KOH (0.2 ml) was stirred at room temp. for 24 h. The reaction mixture was dissolved in $CHCl_3$ and passed through a silica gel column to remove KOH by eluting with $CHCl_3$–MeOH (1 : 1). Recrystallization from MeOH–$CHCl_3$ gave **1** (33 mg) as colorless crystals, which were identical with the naturally occurring glycoside **1** on the basis of IR and ^{1}H-NMR spectral and specific rotation comparisons and mixed melting point determination.

Alkaline Hydrolysis of 6——Treatment of **6** (59 mg) by the same procedure as in the case of **3** gave **7** (38 mg) as colorless crystals, recrystallized from MeOH–$CHCl_3$, mp 90—91 °C. *Anal.* Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 50.81; H, 5.73; N, 4.13. IR ν_{max}^{KBr} cm^{-1}: 3340, 1610, 1555, 1380. ^{1}H-NMR (in C_5D_5N) δ: 5.88 (1H, d, J=3 Hz, $C_{1'}$-H).

***O*-Methylthalicberine**——Colorless needles from EtOH, mp 186—187 °C. High-resolution MS m/z: M^+ Calcd for $C_{38}H_{42}N_2O_6$, 622.3042. Found: 622.3020. IR ν_{max}^{KBr} cm^{-1}: 1610, 1580, 1510, 1260, 1220, 1130. ^{1}H-NMR (in $CDCl_3$) δ: 2.08, 2.52 (each 3H, s, 2 × NCH_3), 3.60, 3.74, 3.82, 3.84 (each 3H, s, 4 × OCH_3). Identical with an authentic sample.[10]

Sugars——Rhamnose, glucose and sucrose were detected by GLC [column: 3% SE-52 on Chromosorb W (AW), temp.: 100→200 °C (program rate, 2 °C/min)] as their trimethylsilylated derivatives.

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