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ON THE CONSTITUENTS OF THE LEAVES OF *VIBURNUM URCEOLATUM*

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Abstract

From the leaves of *Viburnum urceolatum* α-amyrin palmitate, lupeol palmitate, β-amyrin acetate, ursolic acid and β-sitosteryl-β-D-glucoside have been isolated. The flavonol glucoside which had been assigned populnin was revised as astragalin.

Introduction

The deciduous shrub *Viburnum urceolatum* is widely distributed in the mountains of Japan and its leaves are remarkably bitter. Recently, we have isolated four bitter iridoid and bis-iridoid glucosides [1] and a bitter monoterpene diglycoside [2] from the leaves of the plant. The isolation and identification of two flavonol glucosides were described in a previous paper [3]. However, one of the flavonol glucosides **6** which had been assigned populnin was revised as astragalin. Further investigations on the constituents of the plant led the isolation of five known compounds **1**, **2**, **3**, **4** and **5** according to the isolation procedure shown in Fig. 1.

Results and Discussion

Compound **1** which was obtained as a waxy solid gave a positive Liebermann-Burchard's test. The IR spectrum showed the presence of an ester carbonyl at 1740 cm^{-1}, a double bond at 1640 cm^{-1} and a long methylene group at 720 cm^{-1}. The ^1H NMR spectrum indicated signal patterns characteristic of a triterpenoid structure at δ 0.45–3.95 as well as aliphatic straight methylene protons at δ 1.29 (*s*).

Alkaline hydrolysis of **1** gave an alcohol **7**, mp 186° and an aliphatic acid. The IR spectrum of the alcohol **7** showed a hydroxyl and an olefinic absorptions at 3300 and 1640 cm^{-1}, respectively. The IR spectrum was identical with that of α-amyrin. On the other hand, the aliphatic acid was inferred to be palmitic acid from appearance of the fragmetation at *m/z* 256 $[M]^+$, 241, 227 and 213 in the mass spectrum.

Therefore, compound **1** must be α-amyrin palmitate, which was also isolated from *V. phlebotrichum* [4].

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Compound **2** was isolated as needles, mp 78–79°, *m/z* 665 $[M+1]^+$. The IR spectrum showed absorption bands at 1740 cm^{-1} and 1655 cm^{-1} due to an ester carbonyl and a double bond, respectively.

On alkaline hydrolysis, compound **2** afforded an alcohol **8**, mp 215°, $C_{30}H_{50}O$ and an aliphatic acid whose mass spectrum was in good agreement with that of palmitic acid. The IR spectrum of the alcohol **8** revealed the presence of a newly produced hydroxyl group at 3350 cm^{-1} and a terminal double bond at 1640 and 880 cm^{-1}. Signals at *ca* δ 0.75–2.30 in the ^1H NMR spectrum suggested that compound **8** was a triterpenoid. The ^1H NMR spectrum also showed signals of isopropenyl protons at δ 1.67 (3H, *s*), 4.56 (1H, *m*) and 4.66 (1H, *m*) and a proton attached to a carbon bearing the hydroxyl group at δ 3.19 (1H, *m*), besides those of methyl protons at δ 0.75–1.03 (3 H×6). These results suggested that compound **8** was to be a lupeol. Its identity was established by comparison of the IR spectrum with that of an authentic sample. Compound **2** is therefore lupeol palmitate [5].

Compound **3** which was isolated as needles mp 237° had the molecular formula $C_{32}H_{52}O_2$. The IR spectrum showed absorption bands of an ester carbonyl at 1740 and 1250 cm^{-1} and an olefinic group at 1600 cm^{-1}. The ^1H NMR spectrum indicated the presence of acetoxyl protons at δ 2.03 (3H, *s*), an olefinic proton at δ 4.39 (1H, *m*) and a proton attached to a carbon bearing an ester linkage at δ 5.18 (1H, *m*). Prominent peaks at *m/z* 468 $[M]^+$, 218, 203 and 189 in the mass spectrum, arising from a retro-Diels-Alder reaction, suggested that compound **3** had a C–12 unsaturated oleanane or ursane skeleton. The IR spectrum of **3** was superimposable with that of β-amyrin acetate.

Compound **4** was obtained as a powder, mp 227–228°, *m/z* 456 $[M]^+$. The IR spectrum showed absorption bands for a hydroxyl gorpu at 3400 cm^{-1} and a carboxyl gorup at 2550–2350 and 1690 cm^{-1}, which was identical with that of ursolic acid.

Compound **5** was obtained as a white mass, mp 285° with molecular formula $C_{35}H_{60}O_6 \cdot H_2O$. It gave a positive Liebermann-Burchard's reaction. The IR spectrum had a strong hydroxyl band and an olefinic band at 3400 and 1630 cm^{-1}, respectively.

On acetylation with acetic anhydride and pyridine, compound **5** afforded needles **9**, mp 169.5° whose ^1H NMR spectrum showed the presence of four acetoxyl groups at δ 1.96–2.04 (3H×4 *s*).

Hydroloysis of **5** with sulfuric acid gave an alcohol **10**, mp 135° and D-glucose which was confirmed by paper chromatography. The IR spectrum of the alcohol **10** contained a hydroxyl band at 3450 cm^{-1} and an olefinic band at 1640 cm^{-1}, which was in good agreement with that of β-sitosterol.

The coupling constant $J=8$ Hz at δ 5.23 in the ^1H NMR spectrum of **5** suggsted that the glucose was in the β-configuration. The above data showed that 5 was β-sitosteryl-β-D-glucoside. The IR spectra of **5** and **9** were identical with those of authentic samples [6].

Compound **6** was isolated as yellow needles, mp 178° with molecular formula C_{21}

$H_{10}O_{11}\cdot 2H_2O$. Compound **6** had been identified as populnin (kaempferol–7–O–β–D-glucoside) on the chemical and spectral data [3]. Then the position of the glucose was determined to be located at 7-position, since in the UV spectrum of **6** the absorption at 264 nm suffered bathochromic shift of only 2 nm with sodium acetate. However, the position of the glucose was corrected to be 3-position as follows.

Methylation of **6** with the Puride method followed by hydrolysis with hydrochloric acid yielded pale yellow needles **11**, mp 147–149°. It gave a purplish brown color with ferric chloride solution. The ^{1}H NMR spectrum of **11** exhibited two singlets at δ 3.93 (3H) and 3.98 (3H$\times$2) for three methoxyl groups. Two doublets at δ 6.48 and 6.73 (1H each, J=2 Hz) could be assigned to the protons at 6–and 8-positions, respectively. In addition, two doublets appeared at δ 7.13 and 8.37 (2H each, J=10 Hz), which was characteristic for a *p*-substituted B-ring. In the UV spectrum of **11** the absorption at 256 nm suffered no shift with sodium acetate. The absorption at 355 nm shifted to 390 nm on addition of sodium methoxide. Thus, two of three methoxyl groups were located at 4′- and 7-positions. The melting point and color reaction of **11** were in good accord with those of 4′, 5, 7-tri-*O*-methoxylflavonol [7]. Compound **6** was therefore assumed to be astragalin (kameferol–3–*O*–β–D–glucoside). The IR spectrum of **6** was identical with that of astragalin [8].

EXPERIMENTAL

Extraction and isolation. Plant material was collected in Miyazaki prefecture and identified by Dr. S. Sako (Herbarium sample No. 26132). The fresh leaves of *V. urceolatum* Sieb. et Zucc. (2.1 Kg) were extracted with MeOH (10*l*. $\times$2). The combined MeOH solns were concd to dryness to afford a dark green residue (210 g). The residue was diluted with H_2O and extracted with Et_2O. The Et_2O extract (30 g) was chromatographed on Si gel and eluted with $CHCl_3$-MeOH. The fractions eluted with $CHCl_3$ were combined and rechromatographed on Si gel with hexane to give **1** (1.2 g), **2** (64 mg) and **3** (9 mg) in order of polarity.

Compound **1**, a waxy solid; IR ν_{max}^{nujol} cm^{-1}: 1740, 1640, 720; ^{1}H NMR ($CDCl_3$):

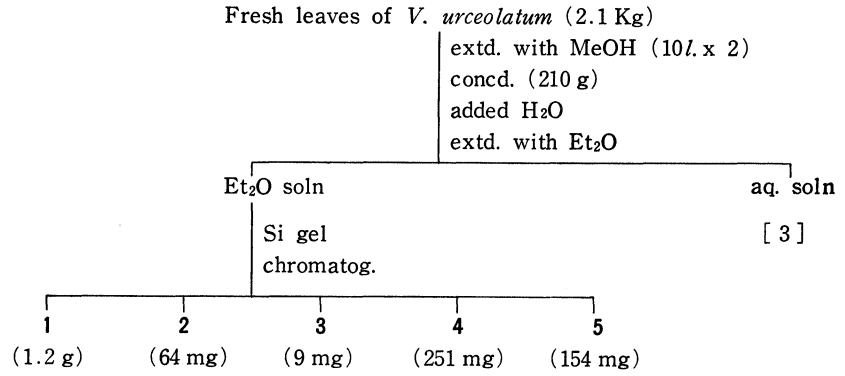
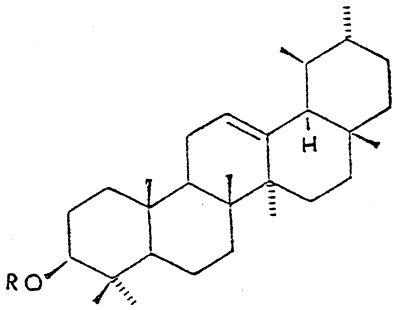
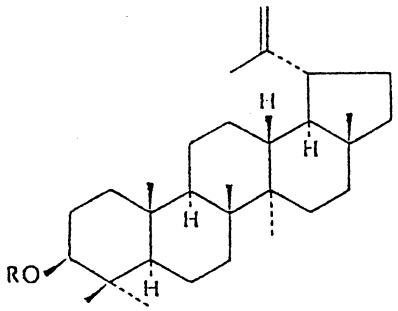


Fig. 1. Isolation of the compounds.



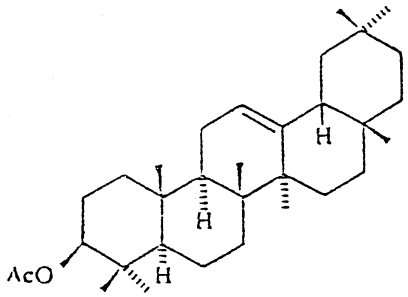
1 R=CO(CH$_2$)$_{14}$CH$_3$

7 R=H

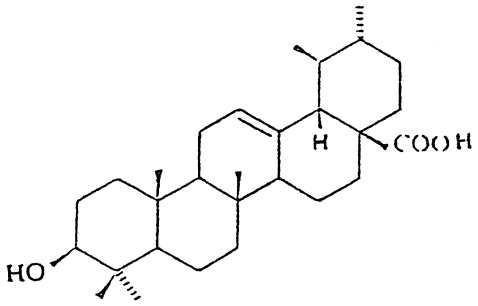


2 R=CO(CH$_2$)$_{14}$CH$_3$

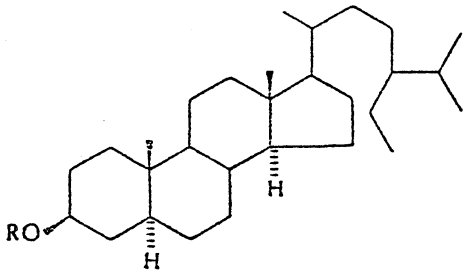
8 R=H



3



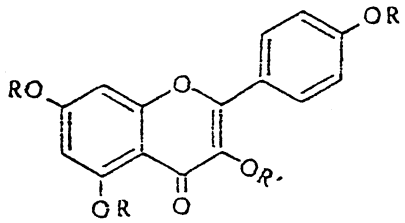
4



5 R=Glc(OH)$_4$

9 R=Glc(OAc)$_4$

10 R=H



6 R=H R'=Glc(OH)$_4$

11 R=Me R'=H

(*s*), 4.77 (1H, *m*), 5.18 (1H, *m*); MS *m/z*: 655 $[M+1]^+$. The IR spectrum was identical with that of α-amyrin palmitate.

Compound **2**, needles from MeOH, mp 78–79° (lit. [5] mp 80–81.5°); IR ν_{max}^{nujol} cm^{-1}: 1740, 1655, 900; ^1H NMR ($CDCl_3$): δ 0.81, 0.86, 0.96, 1.06 (3H×6), 1.29 (*s*), 1.68 (3H, *s*), 4.33 (1H, *m*), 4.58 (1H, *m*), 4.68 4.68 (1H, *m*); MS *m/z*: 665 $[M+1]^+$.

Compound **3**, needles from MeOH-$CHCl_3$, mp 237° (lit. [9] mp 236°); IR ν_{max}^{nujol} cm^{-1}: 1740, 1660, 1250; ^1H NMR ($CDCl_3$): δ 0.84, 0.88, 0.98 (3H × 8), 2.03 (3H, *s*), 4.39 (1H, *m*), 5.18 (1H, *m*); MS *m/z*: 468 $[M]^+$, 453, 408, 218, 203, 189. The IR spectrum was identical with that of β-amyrin acetate.

Elution with $CHCl_3$-MeOH (95:5) gave **4** (251 mg), an amorphous powder from MeOH, mp 227–228° (lit. [9] mp 285–288); IR ν_{max}^{nujol} cm^{-1}: 3400, 2550–2350, 1690; MS *m/z*: 456 $[M]^+$, 438, 248, 207, 189. The IR spectrum was identical with that of ursolic acid.

Elution with $CHCl_3$-MeOH (90:10) gave **5** (154 mg), a white mass from MeOH, mp 285° (lit. [6] mp 250–255°, 280–298°), IR ν_{max}^{nujol} cm^{-1}: 3400, 1630; ^1H NMR (C_5D_5N): δ 5.23 (1H, *d*, *J*=8 Hz), 5.83 (1H, *m*); MS *m/z*: 414 [M-glucose]$^+$. (Found: C, 71.83; H, 10.58% Calc. for $C_{35}H_{60}O_6 \cdot H_2O$: C, 71.75: H, 10.49%.) The IR spectrum was identical with that of β-istostery-β-D-glucoside.

Alkaline hydrolysis of **1**. Compound **1** (80 mg) was dissolved in EtOH (20 ml) and to this soln was added 1N NaOH (0.5 ml). After refluxing for 2 hr, the reaction soln was extracted with Et_2O. Recrystallization of the crude product from acetone afforded needles (18 mg), mp 186° (lit. [9] mp 186°); IR ν_{max}^{nujol} cm^{-1}: 3300, 1640; MS *m/z*: 426 $[M]^+$, 218, 203, 189. The IR spectrum was identical with that of α-amyrin.

The aq. soln was acidified with dil. HCl and extracted with Et_2O to give white scales, mp 50° (lit. [9] mp 63–64°); MS *m/z*: 256 $[M]^+$, 241, 227, 213. The MS spectrum was in accord with that of palmitic acid.

Alkaline hydrolysis of **2**. To a soln of **2** (110 mg) in EtOH (15 ml), was added 2N NaOH (5 ml) and the soln was refluxed overnight. The reaction soln was extracted with Et_2O. The crude product was rectystallied from EtOH to give needles **8** (40 mg), mp 215° (lit. [9] mp 215°); IR ν_{max}^{nujol} cm^{-1}: 3350, 1640, 880; ^1H NMR ($CDCl_3$): δ 0.75, 0.77, 0.82, 0.95, 1,03 (3H×6), 1.67 (3H, *s*), 3.19 (1H, *m*), 4.56 (1H, *m*), 4.66 (1H, *m*); MS *m/z*: 426 $[M]^+$, 408, 393, 365. (Found: 426.3838. Calc. for $C_{30}H_{50}O$: 426. 3860.) The IR spectrum was identical with that of lupeol. The aq. soln was neutralized with dil. HCl and extracted with Et_2O to give palmitic acid (3 mg); MS *m/z*: 256 $[M]^+$, 241, 227, 213. The Mass spectrum was in good agreement with that of plamitic acid.

Acetylation of **5**. Compound **5** (50 mg) was acetylated with acetic anhydride in pyridine. Work-up in the usual way yielded **9** (33 mg), needles from EtOH, mp 169.5°, (lit. [6] mp 168–169°), IR ν_{max}^{nujol} cm^{-1}: 1765, 1230; ^1H NMR ($CDCl_3$): δ 1.96, 2.00,

2.02, 2.04 (3H×4, *s*), 3.20–5.20 (8H, *m*), 5.36 (1H, *m*); MS *m/z*: 745 $[M+1]^+$. The IR spectrum was identical with that of β-sitosteryl-β-D-glucoside acetate.

Hydrolysis of **5**. To a soln of **5** (60 mg) in EtOH (80 ml) was added 2N HCl (5 ml) and the soln was refluxed for 10 hr. The reaction mixture was concd, diluted with H_2O and extracted with Et_2O. Chromatography of the crude product on Si gel with $CHCl_3$-MeOH (98:2) gave **10** (25 mg), plates from $CHCl_3$-MeOH, mp 135° (lit. [9] mp 140°; IR ν_{max}^{nujol} cm^{-1}: 3450, 1640. The IR spectrum was identical with that of β-sitosterol. The aq. soln was neutralized with excess of $BaCO_3$, the precipitate was filterd off and the filtrate was evaporated to dryness *in vacuo*. The presence of D-glucose in the residue was confirmed by paper chromatography.

Methylation of **6** *followed by acid hydrolysis*. A soln of **6** (84 mg) in DMF (1 ml) was treated with Ag_2O (200 mg) and MeI (1 ml) and the reaction mixture was stirred at 5° for 3 days. After usual work-up, the crude product was chromatographed on Si gel with $CHCl_3$-MeOH (99:1) to give the methylate (84 mg), IR ν_{max}^{nujol} cm^{-1}: 1630, 1610, 1520, 840. To a soln of the methylate (84 mg) in MeOH (0.5 ml), was added 2N HCl (0.5 ml) and the mixture was refluxed for 3 hr. The resulting precipitate was filterd and recrystallized from MeOH to afford **11** (30 mg), pale yellow needles, mp 147–149° (lit. [7] mp 149–150°), IR ν_{max}^{nujol} cm^{-1}: 3550, 1610, 1520, 1500, 840; ^1H NMR ($(CD_3)_2CO$): δ 3.93 (3H, *s*), 3.98 (3H × 2, *s*), 6.48, 6.75 (1H each, *d*, J=2 Hz), 7.13, 8.37 (1H each, *d*, J=10 Hz); UV λ_{max}^{MeOH} nm: 256, 355; $\lambda_{max}^{MeOH\text{-}NaOAc}$ nm: no shift; $\lambda_{max}^{MeOH\text{-}NaOMe}$ nm: 258, 390. It gave a purplish brown color with ferric chloride solution.

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