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PORTULOSIDE A, A MONOTERPENE GLUCOSIDE, FROM *PORTULACA OLERACEA*

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Key Word Index—*Portulaca oleracea*; Portulacaceae; portuloside A; monoterpene glucoside; linalool tetraacetylglucoside; photooxygenation.

Abstract—From the methanol extract of *Portulaca oleracea*, a new monoterpene glucoside, portuloside A, has been isolated. The structure of portuloside A was established by spectroscopic methods and then confirmed to be (3*S*)-3-(3,7-dimethylocta-1,7-dien-6-onyl)-β-D-glucopyranoside by synthesis from linalool.

INTRODUCTION

In the course of our search for biologically active natural products [1], the methanol extract of the aerial parts of *Portulaca oleracea* was found to exhibit moderate antimicrobial activity against *Bacillus subtilis*. In the course of isolating the active substance, we isolated a new monoterpene glucoside (**1**) which we have named portuloside A. This paper deals with the isolation, structural elucidation and synthesis of this new compound.

RESULTS AND DISCUSSION

The methanol extract of *P. oleracea* was partitioned between ethyl acetate and water. The ethyl acetate-soluble portion retained the antimicrobial activity. Repeated column chromatography gave a monoterpene glucoside (**1**) which was isolated as its acetylated derivative **1a** and then recovered by alkaline hydrolysis. The acetylated derivative **1a** was used for structural elucidation.

The molecular formula of compound **1a** was established as $C_{24}H_{34}O_{11}$ by HRCI-mass spectrometry (*m/z* 499.2168 $[M+H]^+$). The NMR spectra (Table 1) showed the presence of two tertiary methyl groups at δ_H 1.35 (3H, *s*, H_3-10) and 1.88 (3H, *s*, H_3-9), the latter of which was long-range coupled to signals at δ_H 5.75 and 5.97 due to an exomethylene, thereby forming a partial unit (C-7–C-9) as shown in Fig. 1, as well as of a ketone function at δ_C 201.77 (C-6) and an oxygen-bearing quaternary carbon at δ_C 80.02 (C-3). In addition, the 2D H-H COSY and HMQC data indicated the presence of a monosubstituted double bond (C-1–C-2), a CH_2CH_2 unit and a tetraacetylglucopyranosyl moiety. The configuration of the anomeric position was assigned to β based on the large *J* value (8.1 Hz) of the anomeric proton signal. The structural fragments were assembled by means of an HMBC experiment, the results of which are summarized in Fig. 1. Consequently, the aglycone part was clarified to be a variant of linalool (Fig. 1). The anomeric proton signal at δ_H 4.57 showed a three-bond correlation with the sole quaternary carbon signal (C-3) on HMBC, resulting in the connection of the glucosyl moiety to the C-3 position in the aglycone. The above spectral data are consistent with the structure **1a**. Accordingly, the structure of portuloside A (**1**) is therefore, 3-(3,7-dimethylocta-1,7-dien-6-onyl)-β-D-glucopyranoside. The configuration of the C-3 chiral centre, however, remained equivocal. In order to establish its chirality unambiguously, synthesis of portuloside A with 3*R* or 3*S* configurations was attempted starting from linalool. Glycosylation of (−)-linaloo with 2,3,4,6-*O*-tetraacetyl-D-glucopyranosyl trichloroacetimidate in the presence of boron trifluoride etherate [2] gave linalyl tetracetylglucoside (**4**) [3–6], which was subjected to photooxygenation under the conditions reported by Mihelich and Eickhoff [7] to yield the 3*R* form **2a** in 19% yield, the spectra data of which were not consistent with those of **1a**. This result indicates that **1a** should have 3*S* configuration. Since the (+)-linalool needed to synthesize **1a** was not available, (±)-linalool was glycosylated as for the preparation of **4**, giving rise to a diastereomeric mixture of **3** and **4**, which was photo-oxygenated without separation and then purified by HPLC to afford two diastereomers. One was the same as **2a** and the one, which must be 3*S*, was identical in all respects with **1a**. The spectral data and specific rotation of the product obtained on hydrolysis of the synthetic **1a** were identical with those of **1**. Thus,

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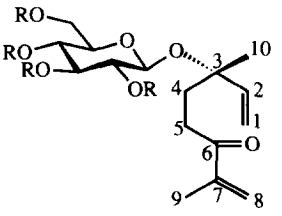

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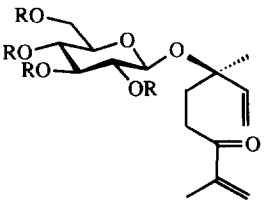
1a R = Ac



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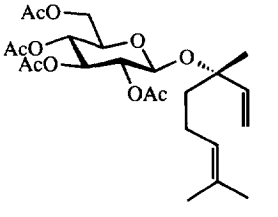

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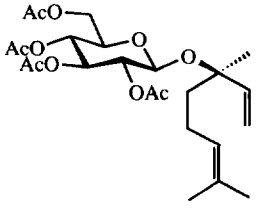

4

Table 1. ^{13}C NMR (150 MHz) data of compounds **1**, **1a** and **2**

C	1*	1a†	2*
1	114.90	115.98	114.24
2	144.11	141.21	144.71
3	79.54	80.02	79.50
4	36.22	35.60	34.52
5	32.82	31.85	32.72
6	202.18	201.79	202.43
7	144.28	144.28	144.36
8	124.65	124.65	124.69
9	17.79	17.64	17.81
10	24.25	23.82	25.05
G-1	99.63	95.96	99.39
G-2	75.38	71.36	75.29
G-3	78.81	71.60	78.80
G-4	71.87	68.73	71.84
G-5	78.11	72.91	78.18
G-6	62.97	62.31	62.97
$\underline{C}OCH_3$		169.31	
		169.46	
		170.28	
		170.59	
$CO\underline{C}H_3$		17.65	
		20.61	

*In C_5D_5N.
†In $CDCl_3$.

the structure of portuloside A (**1**) was determined as (3*S*)-3-(3,7-dimethylocta-1,7-dien-3-onyl)-β-D-glucopyranoside.

Portuloside A exhibited no antimicrobial activity.

EXPERIMENTAL

^1H and ^{13}C NMR: TMS as int. standard; CC: silica gel (Merck, 230–400 mesh); TLC: precoated silica gel F254 (Merck). Spots were visualized by UV (254 nm) and 10% $CeSO_4$–H_2SO_4.

Plant material. Aerial parts of *P. oleracea* L. were collected in Tokushima, Japan. A voucher specimen has been deposited in our institute.

Extraction and isolation. The MeOH extract was partitioned between EtOAc and water. The EtOAc-soluble portion (24.4 g) was chromatographed by CC on silica gel eluted in turn with $CHCl_3$, 1% MeOH in $CHCl_3$, 5% MeOH in $CHCl_3$, 10% MeOH in $CHCl_3$, 20% MeOH in $CHCl_3$ to give 14 frs (1–14). Fr. 12 (1.9 g) was again chromatographed by CC on silica gel with 20% MeOH in $CHCl_3$ to give 21 frs (14–34). Fr. 24 (319 mg) was acetylated by Ac_2O–pyridine to give the crude product (245 mg), which was purified by

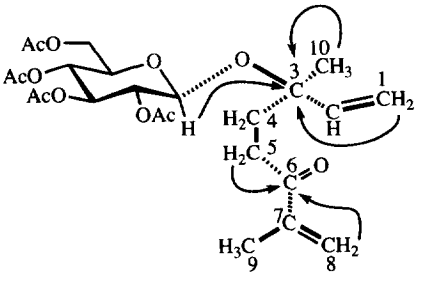

Fig. 1. Representative HMBC correlations (arrows) and partial structural fragments (bold lines) for **1a**.

repeated CC on silica gel with *n*-hexane–Me_2CO (3:1) to give portuloside A tetraacetate (**1a**) (4.5 mg).

Portuloside A tetraacetate (**1a**). Oil, $[\alpha]_D^{24}$ −12.1° (*c* 0.45, $CHCl_3$). HR-CI-MS *m/z*: 499.2168 $[M+H]^+$ (calc. 499.2179 for $C_{24}H_{35}O_{11}$); ^1H NMR (600 MHz, $CDCl_3$): δ 1.35 (3H, *s*, H_3-10), 1.86 (2H, *m*, H_2-4), 1.88 (3H, *s*, H_3-9), 2.00 (3H, *s*, Ac), 2.01 (3H, *s*, Ac), 2.04 (3H, *s*, Ac), 2.05 (3H, *s*, Ac), 2.63 (1H, *ddd*, *J* = 17.0, 10.7, 5.3 Hz, H-5), 2.86 (1H, *ddd*, *J* = 17.0, 10.9, 5.1 Hz, H-5), 3.64 (1H, *ddd*, *J* = 8.1, 5.7, 2.4 Hz, G-5), 4.06 (1H, *dd*, *J* = 12.0, 2.4 Hz, G-6), 4.17 (1H, *dd*, *J* = 12.0, 5.7 Hz, G-6), 4.57 (1H, *d*, *J* = 8.1 Hz, G-1), 5.00 (1H, *t*, *J* = 8.1 Hz, G-2), 5.02 (1H, *d*, *J* = 10.0, 1.0 Hz, H-1), 5.20 (1H, *t*, *J* = 8.1 Hz, G-3), 5.22 (1H, *t*, *J* = 8.1 Hz, G-4), 5.22 (1H, *t*, *J* = 17.8, 1.0 Hz, H-1), 5.68 (1H, *dd*, *J* = 17.8, 10.0 Hz, H-2), 5.75 (1H, *s*, H-8), 5.97 (1H, *s*, H-8); ^{13}C NMR: Table 1.

Synthesis of (3R)-*portuloside A tetraacetate* (**2a**). To a soln of D-glucopyranosyl trichloroacetimidate (1.2 g) in dry CH_2Cl_2 (8 ml) was added (−)-linaloo (0.7 ml) at room temp. and the mixt. was cooled to 0°. A soln of $BF_3 \cdot OEt_2$ (0.25 ml) in CH_2Cl_2 (2.0 ml) was added dropwise over 20 min, and stirring continued for 2.5 hr. After addition of K_2CO_3 and CH_2Cl_2, the mixt. was washed with H_2O and satd $NaHCO_3$ soln, filtered and the filtrate was evapd *in vacuo*. The residue (1.7 g) was chromatographed on silica gel (*n*-hexane–acetone Me_2CO, 4:1) to yield (3*R*)-linalyl β-D-glucopyranoside tetraacetate (**4**) (340 mg) as an oil. The immersion-well reactor was filled with a soln of **4** (144 mg), *meso*-tetraphenylporphine (TPP, 0.2 mg), dimethylaminopyridine (0.11 ml), Ac_2O (1 ml) and pyridine (6 ml) in CH_2Cl_2 (8 ml). Oxygen was bubbled through the soln for 5 min, and the mixt. cooled with ice–water and irradiated with a 400 W high-pressure mercury immersion lamp while the passage of O_2 was continued for 1.5 hr. The solvent was evapd *in vacuo* and residue chromatographed on silica gel with toluene–EtOAc (5:1) to give (3*R*)-portuloside A tetraacetate (**2a**). $[\alpha]_D^{24}$ +3.9° (*c* 0.76, $CHCl_3$). HR-CI-MS *m/z*: 499.2161 $[M+H]^+$ (calc. 499.2179 for $C_{24}H_{35}O_{11}$); ^1H NMR (600 MHz, $CDCl_3$): δ 1.25 (3H, *s*, H_3-10), 1.86 (3H, *s*, H_3-9), 1.91 (2H, *m*, H_2-4), 1.99 (3H, *s*, Ac), 2.01 (3H, *s*, Ac), 2.04 (3H, *s*, Ac), 2.05 (3H, *s*, Ac), 2.68 (1H, *ddd*, *J* = 17.0, 10.4, 5.2 Hz, H-5), 2.85 (1H, *ddd*, *J* = 17.0, 10.0, 5.1 Hz, H-5), 3.60 (1H, *ddd*, *J* = 8.1, 5.3, 2.5 Hz, G-5), 4.03 (1H, *dd*, *J* = 12.1, 2.5 Hz, G-6), 4.12 (1H, *dd*, *J* = 12.1, 5.3 Hz, G-6), 4.53 (1H, *d*, *J* = 8.1 Hz, G-1), 5.00 (1H, *t*, *J* = 8.1 Hz, G-2), 5.00 (1H, *t*, *J* = 8.1 Hz, G-4), 5.20 (1H, *t*, *J* = 8.1 Hz, G-3), 5.20 (1H, *d*, *J* = 17.5 Hz, H-1), 5.76 (1H, *s*, H-8), 5.87 (1H, *dd*, *J* = 17.5, 11.0 Hz, H-2), 5.95 (1H, *s*, H-8).

Synthesis of (3S)-*portuloside A tetraacetate* (**1a**). To a soln of D-glucopyranosyl trichloroacetimidate (10.0 g) in dry CH_2Cl_2 (89 ml) was added (±)-linalool (7.7 ml) at room temp. and the mixt. was cooled to 0°. A soln of $BF_3 \cdot OEt_2$ (3 ml) in CH_2Cl_2 (22 ml) was added dropwise in 20 min, and stirring continued for 1 hr. After addition of K_2CO_3 and CH_2Cl_2, the reaction mixt. was washed with H_2O and satd $NaHCO_3$ soln, filtered and the filtrate evapd *in vacuo*. The residue (17 g) was chromatographed on silica gel (*n*-hexane–Me_2O, 4:1) to yield a mixture of (3*S*)- and (3*R*)-linalyl β-D-glucopyranoside tetraacetates (1.6 g). This diastereomeric mixture (1.2 g) was transferred into the immersion-well reactor filled with a soln of TPP (2 mg), dimethylaminopyridine (0.7 ml), Ac_2O (3 ml), and pyridine (10 ml) in CH_2Cl_2 (20 ml). Oxygen was bubbled through the soln for 5 min, and the mixt. was cooled with ice–water and irradiated with a 400 W high-pressure mercury immersion lamp while the passage of O_2 was continued for 1.5 hr. The solvent was removed *in vacuo*, the residue chromatographed on silica gel with toluene–EtOAc (5:1) followed by HPLC [8 times recycled; JAIGEL-1H (φ20 × 600 mm), $CHCl_3$ (3.5 ml min^{-1})] to give (3*S*)-portuloside A tetraacetate (**1a**) (10 mg) and (3*R*)-portuloside A tetraacetate (**2a**) (15 mg). **1a**: $[\alpha]_D^{24}$ −8.5° (*c* 0.42, $CHCl_3$). HR-CI-MS *m/z* (rel. int.): 499.2157 $[M+H]^+$ (calc. 499.2179 for $C_{24}H_{35}O_{11}$); ^1H and ^{13}C NMR spectra were identical with those of the natural product.

Alkaline hydrolysis of (3S)-*portuloside A tetraacetate* (**1a**). To a soln of a mixture of **1a** (10 mg) in THF (4 ml) was added 0.5 N NaOH (0.6 ml) and stirring continued at room temp. for 1.5 hr. The solvent was removed and the residue was taken up in EtOAc. The organic layer was washed with satd NaCl soln, dried over $MgSO_4$, filtered and the filtrate was evapd *in vacuo*. The residue was purified by HPLC [Cosmosil 5C18 (φ10 × 250 mm), 40% H_2O in MeOH (2.5 ml min^{-1})] to give (3*S*)-portuloside A (**1**) (5.0 mg) as an oil, $[\alpha]_D^{24}$ −19.6° (*c* 0.50, $CHCl_3$). HR-FAB-MS *m/z*: 353.1591 $[M+Na]^+$ (calc. 353.1576 for $C_{16}H_{26}O_7Na$); ^1H NMR (600 MHz, C_5D_5N): δ 1.54 (3H, *s*, H_3-10), 1.82 (3H, *s*, H_3-9), 2.11 (1H, *dt*, *J* = 14.0, 7.9 Hz, H-5), 2.15 (1H, *dt*, *J* = 14.0, 7.9 Hz, H-5), 3.02 (2H, *t*, *J* = 7.9 Hz, H_2-4), 3.81 (1H, *m*, G-5), 3.97 (1H, *m*, G-6), 4.17 (2H, *m*, G-3, 4), 4.27 (1H, *dd*, *J* = 7.8, 7.8 Hz, G-2), 4.43 (1H, *dd*, *J* = 11.7, 2.7 Hz, G-6), 4.93 (1H, *d*, *J* = 7.8 Hz, G-1), 5.17 (1H, *d*, *J* = 11.0 Hz, H-1), 5.40 (1H, *d*, *J* = 17.6 Hz, H-1), 5.58 (1H, *s*, H-8), 5.91 (1H, *s*, H-8), 6.22 (1H, *dd*, *J* = 17.6, 11.0 Hz, H-2); ^{13}C NMR: Table 1.

Alkaline hydroylsis of (3R)-*portuloside A tetraacetate* (**2a**). Treatment of **2a** (12 mg) in an identical manner to that just described for **1a** gave (3*R*)-portuloside A (**2**) (5.6 mg) as an oil, $[\alpha]_D^{24}$ −11.5° (c 0.56, $CHCl_3$). HR-CI-MS *m/z*: 331.1734 $[M+H]^+$ (calc. 331.1757 for $C_{16}H_{27}O_7$); ^1H NMR (600 MHz, C_5D_5N): δ 1.48 (3H, *s*, H_3-10), 1.85 (3H, *brs*, H_3-9), 2.20 (2H, *t*, *J* = 8.3 Hz, H_2-4), 3.05 (1H, *dt*, *J* = 18.1, 8.3 Hz, H-5), 3.19 (1H, *dd*, *J* = 18.1, 8.3 Hz, H-5), 3.80 (1H, *m*, G-5), 3.96 (1H, *m*, G-6), 4.17 (2H, *m*, G-3, 4), 4.25 (1H, *dd*, *J* = 8.0, 8.0 Hz, G-2), 4.40 (1H, *dd*, *J* = 11.7, 2.7 Hz, G-6), 4.91 (1H, *d*, *J* = 8.0 Hz, G-1), 5.14 (1H, *d*, *J* = 11.0 Hz, H-1), 5.33 (1H, *d*, *J* =

17.6 Hz, H-1), 5.60 (1H, *brs*, H-8), 5.98 (1H, *brs*, H-8), 6.29 (1H, *dd*, $J = 17.6$, 11.0 Hz, H-2); ^{13}C NMR: Table 1.

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