A New Resin Glycoside, Muricatin IX, from the Seeds of Ipomoea muricata

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A new resin glycoside, named muricatin IX (I), was isolated from the seeds of Ipomoea muricata (L.) Jacq. (Convolvulaceae). The structure of I was determined on the basis of spectroscopic data as well as chemical evidence. Compound I is the first representative of resin glycosides in which an organic acid connects the sugar moiety and the aglycone moiety to form macrocyclic ester ring.

Key words resin glycoside; Ipomoea muricata; Convolvulaceae; muricatin

The seeds of Ipomoea muricata (L.) Jacq. (Convolvulaceae) are used as a laxative and carminative folk medicine in India.1) In a preceding paper,1) Noda et al. reported that alkaline hydrolysis of the crude resin glycoside fraction of the seeds of I. muricata gives three organic acids, isobutyric, (2R)-methylbutyric, and (3R)-hydroxy-(2R)-methylbutyric ((2R,3R)-nilic) acids, along with a glycosidic acid fraction composed of L-rhamnose, D-fucose, D-quinovose, and (11R)-hydroxyhexa-decanoic ((11R)-jalapinolic) acids, whose absolute configuration was later corrected to be S.2) They also discussed the isolation and structural elucidation of three glycosic acids including muricatic acids A–C.1,3) Further, eight genuine resin glycosides, muricatins I–VIII, which possessed characteristic macrolactone structures, were reported.3,4) As part of an ongoing study of the resin glycosides from Convolvulaceae plants,5) the present report deals with the isolation and structural elucidation of a new resin glycoside, muricatin IX, from the seeds of I. muricata.

The powdered seeds of I. muricata were extracted with methanol (MeOH). The extract was suspended in H2O and successively extracted with ethyl acetate (EtOAc) and n-butanol (BuOH). The EtOAc-soluble fraction was subjected to silica gel chromatography and HPLC on an octadecyl silica column to yield I. Compound I, named muricatin IX, was obtained as an amorphous powder. The negative-ion and positive-ion FAB-MS data of I showed an [M–H]+ ion peak at m/z 937 and an [M+Na]+ ion peak at m/z 961, respectively, indicating its molecular weight to be 938. The molecular formula of I was established as C31H30O30 by high-resolution (HR)-negative-ion FAB-MS. Upon alkaline hydrolysis, I gave an organic acid fraction and a glycosidic acid. Analysis of the organic acid fraction by gas chromatography (GC) revealed the presence of nilic acid. The absolute configuration of nilic acid of the crude resin glycoside fraction of the seeds of I. muricata was previously defined as 2R,3R by comparison of the specific rotation and 1H-NMR spectral data of its methyl ester with those of authentic sample.5) The glycosidic acid was identified as muricatic acid C (2) from its 1H-NMR spectrum.3) The 1H-NMR spectrum of I exhibited signals attributable to one primary methyl group [δ 0.85 (t, J = 7.5 Hz)], one H-2 [δ 2.82 (dq, J = 9.5, 7.5 Hz)] of niloyl residue (Nla), four anomic protons [δ 5.97 (s), 5.82 (d, J = 7.5 Hz), 4.97 (d, J = 8.0 Hz), 4.83 (d, J = 7.5 Hz)], and six secondary methyl groups [δ 1.77 (d, J = 6.5 Hz), 1.60 (d, J = 6.5 Hz), 1.50 (d, J = 5.5 Hz), 1.47 (d, J = 5.5 Hz), 1.29 (d, J = 6.0 Hz), 1.08 (d, J = 7.5 Hz)]. The 13C-NMR spectrum showed signals corresponding to two carboxyl carbons (δ 175.5, 173.4) and four anomic carbons (δ 103.6, 103.0, 102.7, 100.6). From these data, it was elucidated that I is composed of 1 mol each of nilic acid and 2. Furthermore, the molecular formula, and the nonequivalent signals due to H2-2 [δ 2.57 (ddd, J = 6.0, 11.5, 15.5 Hz), 2.47 (ddd, J = 5.0, 11.0, 15.5 Hz)] of the aglycone (11S)-jalapinolic acid moiety (Jla) in the 1H-NMR spectrum, suggested that I has a macrocyclic ester ring. The 1H- and 13C-NMR signals of I were assigned with the aid of 1H–1H correlation spectroscopy (COSY), 1H–1H total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra (Table 1). Comparison of the chemical shifts of the 1H-NMR signals of I and methyl ester (2a)6) of 2 indicated acylation shift (Δδ = δ1–δ2a) of the signal due to H-3 of Jla and H-3 of Nla was shifted downfield by 1.01 ppm as compared with the signal due to H-3 of niloyl residue in muricatin VII (3).3) These data indicated that the ester linkages were located at OH-3 of Quiα and OH-3 of Nla. In the HMBC spectrum of I, key cross-peaks were observed between H-3 of Nla and C-1 of Jla, and between H-3 of Quiα and C-1 of Nla (Fig. 1). Therefore, the ester linkages of Jla and Nla were located at OH-3 of Nla and OH-3 of Quiα, respectively. These linkages were supported by the fragment ion peaks observed at m/z 855 [M–H–82 (niloyl unit–H2O)]−, 791 [937–146 (deoxyhexose unit)]−, 709 [855–146]−, 563 [709–146]−, 417 [563–146]−, and 271 [417–146]− in the negative-ion FAB-MS of I (Fig. 2).

Accordingly, I was defined as (11S)-jalapinolic acid 11-O-α-D-rhamnopyranosyl-(1→2)-[O(3-O-(2R,3R-niloyl))-β-D-quinovopyranosyl-(1→3)]-O-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-1,3(niloyl)olide (Fig. 3).

It should be noted that I is different from all the resin glyco-
cosides isolated so far, in that the carboxyl group of its aglycone moiety linked with a hydroxyl group of the organic acid, which is attached to a sugar moiety by ester linkage, to form a macrocyclic ester ring.

**Experimental**

**General Procedures** Optical rotation was determined with a JASCO P-2300 polarimeter (JASCO, Tokyo, Japan). The $^1$H- and $^{13}$C-NMR spectra were recorded by using a JEOL
ECA-500 spectrometer (JEOL, Tokyo, Japan), and chemical shifts are given on a $\delta$ (ppm) scale with tetramethylsilane (TMS) as an internal standard. MS data were collected using a JEOL JMS-700 mass spectrometer (JEOL). Analytical GC was carried out with a Shimadzu gas chromatograph GC-8A with a flame-ionization detector (Shimadzu, Kyoto, Japan). Column chromatography was carried out over silica gel 60 (Merck, Art. No. 1.07734; Merck, Darmstadt, Germany). HPLC separation was performed on a Shimadzu LC-10AS micro pump with a Shimadzu RID-10A RI detector (Shimadzu).

**Plant Material** The seeds of *I. muricata* were purchased from Takii & Co., Ltd., a seed and sapling company in Kyoto, Japan and identified by one of authors (Prof. Toshihiro Nozohara). A voucher specimen has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

**Extraction and Isolation** The powdered seeds of *I. muricata* (532.2 g) were extracted with MeOH ($500 \text{mL} \times 3$) at room temperature for 18 d, and the solvent was removed under reduced pressure to afford a MeOH extract (72.0 g). The MeOH extract was suspended in H$_2$O (330 mL) and then successively extracted with EtOAc (200 mL, 100 mL $\times 3$) and BuOH (300 mL) to afford a EtOAc-soluble fraction (42.0 g) and a BuOH soluble-fraction (5.4 g). A part (32.0 g) of the EtOAc-soluble fraction was chromatographed on silica gel column using gradient mixtures of CHCl$_3$–MeOH–H$_2$O (1 : 0 : 0.30 : 1 : 0) as eluents to furnish fractions 1–24. HPLC [column 1, COSMOSIL 5C18-AR-II (Nacalai Tesque, Inc., Kyoto, Japan, 20 mm i.d.×250 mm)] of fraction 22 (384 mg) eluted with 90% MeOH gave fractions 22-1–22-13. Fraction 22-12 (51 mg) was subjected to HPLC (column 1) eluted with 85% MeOH to afford 1 (24 mg).

**Muricatin IX (1)**

Amorphous powder, $[\alpha]_D^{20}$ $-$42.4° (c=3.1, MeOH). Positive-ion FAB-MS $m/z$: 961 [M+Na]$^+$. Negative-ion FAB-MS $m/z$: 937 [M−H]$^−$, 855 [937−82]$^−$, 791 [937−146]$^−$, 709 [855−146]$^−$, 563 [709−146]$^−$, 417 [563−146]$^−$, 271 [417−146]$^−$. HR-negative-ion FAB-MS $m/z$: 937.5025 (Caled for $\text{C}_{45}\text{H}_{77}\text{O}_{20}$, 937.5014).

**Alkaline Hydrolysis of 1** A solution of 1 (5mg) in 1m KOH–1,4-dioxane (9:1, 1.0mL) was heated at 95°C for 1h. The reaction mixture was adjusted to pH 3 with 1m HCl and then diluted with H$_2$O (10mL) and extracted with ether (3×3mL). The ether layer was dried over MgSO$_4$ and evaporated to furnish an organic acid fraction. The organic acid fraction was methylated with diazomethane–ether and then the reaction mixture was analyzed by GC [column, Unisole 30T (5%) (GL Sciences Inc., Tokyo, Japan, 3.2 mm i.d.×2m glass column); column temperature, 90°C; carrier gas, N$_2$ 1.2kg/cm$^2$; retention time ($t_R$) (min): 6.28 (methyl nilate)].

The aqueous layer was desalted by Diaion HP20 (Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan) column chromatography (H$_2$O, acetone) to give a glycosidic acid as an amorphous powder (3 mg). The glycosidic acid was identical with 2 by comparison of $^1$H-NMR spectrum with that of authentic sample.

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**Conflict of Interest** The authors declare no conflict of interest.

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