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Ipomotaosides A−D, Resin Glycosides from the Aerial Parts of *Ipomoea batatas* and Their Inhibitory Activity against COX-1 and COX-2

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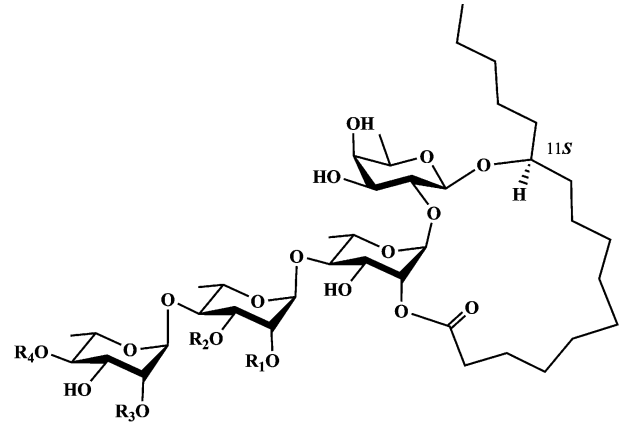
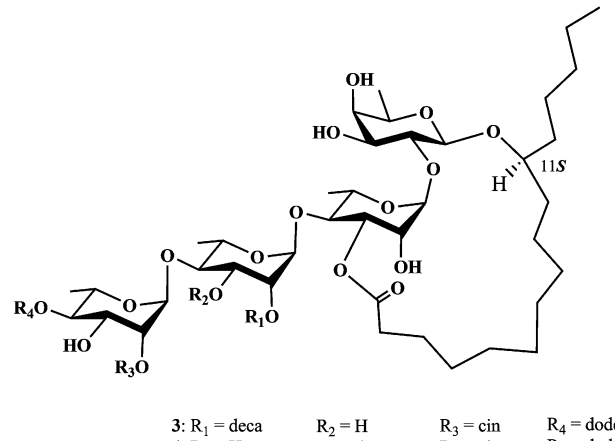
Four new resin glycosides, namely, ipomotaosides A−D (**1**−**4**), were isolated from the dried aerial parts of *Ipomoea batatas*. The structures of **1**−**4** were elucidated by analysis of their spectroscopic data and by chemical derivatization and were tested for their anti-inflammatory activity against cyclooxygenase (COX)-1 and -2.

The rhizomes of *Ipomoea batatas* (L.) Lam. (Convolvulaceae) are used for food and as a folk medicine, and the plant has been cultivated as a health food in western Japan.[2] In mainland China, the roots and leaves have been used in folkloric medicine for anemia, diabetes, hemorrhage, hypertension, and leukemia.[3] The genus *Ipomoea* was shown to be a rich source of resin glycosides, which have been reported from the roots of *I. batatas*.[4−10] However, the aerial parts of this species are disposed of without being used in Japan. In the course of a study on the development of new medicinal resources, a chemical study was initiated of the aerial parts of *I. batatas*. The ethyl acetate extract was fractionated and subjected to column chromatography and subsequent preparative HPLC to afford four new resin glycosides, designated as ipomotaosides A (**1**), B (**2**), C (**3**), and D (**4**). The structures of **1**−**4** were elucidated by various NMR techniques, including COSY, HMQC, HMBC, DEPT, and ROESY experiments and by chemical degradation. The anti-inflammatory activity of **1**−**4** against COX-1 and COX-2 was examined.



1: R_1 = H R_2 = deca R_3 = cin R_4 = dodeca
2: R_1 = dodeca R_2 = H R_3 = cin R_4 = dodeca



3: R_1 = deca R_2 = H R_3 = cin R_4 = dodeca
4: R_1 = H R_2 = deca R_3 = cin R_4 = dodeca

(*n*-decanoyl = deca; *n*-dodecanoyl = dodeca; *trans*-cinnamoyl = cin)

Results and Discussion

A mixture of **1**−**4** was treated with 5% KOH to afford the organic acid components, *n*-decanoic acid (deca), *n*-dodecanoic acid (dodca), and *trans*-cinnamic acid (cin), from the diethyl ether layer. The *n*-BuOH layer furnished a glycosidic acid (**5**), which showed a quasi molecular ion peak at *m*/*z* 879 $[M + Na]^+$ in its HRFABMS, indicative of a molecular formula of $C_{40}H_{72}O_{19}$. On mild acidic hydrolysis, **5** furnished (11*S*)-jalapinolic acid (Jla) (**6**),[11] in addition to D-fucose and L-rhamnose, in a ratio of 1:3, as sugar components. NMR experiments (COSY, HMQC, HMBC, and ROESY) of **5** allowed the full assignments of the sugar units (Tables 1 and 2). Compound **5** was identified as operculinic acid C, obtained previously from *Ipomoea operculata*.[11]

Ipomotaoside A (**1**) gave a $[M + Na]^+$ peak at *m*/*z* 1327.7911 in its HRFABMS, corresponding to a molecular formula of $C_{71}H_{116}O_{21}$, requiring 14 unsaturation equivalents. The IR spectrum of **1** showed absorptions due to hydroxy (3445 cm^{-1}), carbonyl (1715 cm^{-1}), and aromatic (1635 cm^{-1}) functions. The negative FABMS of **1** exhibited fragment ion peaks at *m*/*z* 1149 [M −154 (deca) − H]$^-$, 1019 [1149 − 130 (cin)]$^-$, 837 [1019 − 182 (dodeca)]$^-$, 545, 417, and 271, suggesting that this compound includes one unit each of *n*-decanoic acid, *trans*-cinnamic acid, *n*-dodecanoic acid, and **5**. The diagnostic fragment ion peaks at *m*/*z* 545 [417 + 128 (146 − H_2O)]$^-$, 417 [271 + 146]$^-$, and 271 [Jla − H]$^-$ indicated that a macrocyclic disaccharide ester formed between the second sugar (Rha′) and the carbonyl group of jalapinolic acid (**6**).[12](Moreover, these diagnostic fragment ion peaks were observed in the FABMS of each of **2**−**4**, to be described below.) The ^{1}H NMR spectrum of **1** exhibited three triplet methyls and many methylene signals assignable to the organic acids (Org), a *trans*-cinnamoyl group as confirmed by the distinctive *trans*-coupled olefinic protons at δ 6.61 (d, J = 16.0 Hz) and 7.88 (d, J = 16.0 Hz) and aromatic protons at δ 7.36−7.44 (m, 5H), four acylated protons at δ 5.84 (t, J = 9.9 Hz), 5.85 (dd, J = 9.3, 3.0 Hz), 5.94 (dd, J = 3.2, 1.6 Hz), and 5.95 (dd, J = 2.2, 1.5 Hz), and four anomeric protons at δ 4.77 (d, J = 7.4 Hz), 5.50 (d, J = 1.5 Hz), 5.70 (d, J = 1.6 Hz), and 6.23 (d, J = 1.6 Hz) (Table 1). The chemical shifts and coupling constants of the signals due to the sugar moieties were compatible with a β-linkage for the fucopyranosyl in $^4C_1$ conformation and α-linkages for all the rhamnopyranosyl units in $^1C_4$ conformation. The ^{13}C NMR spectrum

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**5** (in pyridine-d_5, 600 MHz)

	position	1	2	3	4	5
aglycone	11	3.87 (m)	3.84 (m)	3.88 (m)	3.92 (m)	3.89 (m)
	Cin-2	6.61 (d, 16.0)	6.45 (d, 15.9)	6.43 (d, 15.9)	6.56 (d, 15.9)	
	3	7.88 (d, 16.0)	7.75 (d, 15.9)	7.72 (d, 15.9)	7.85 (d, 15.9)	
	5,9	7.36 (m)	7.28 (m)	7.29 (m)	7.34 (m)	
	6,8	7.44 (m)	7.33 (m)	7.31 (m)	7.40 (m)	
	7	7.36 (m)	7.28 (m)	7.29 (m)	7.34 (m)	
	Me	0.82 (t, 7.3)	0.82 (t, 7.3)	0.83 (t, 7.3)	0.83 (t, 7.3)	0.83 (t, 7.3)
	Me	0.86 (t, 7.1)	0.84 (t, 7.3)	0.86 (t, 7.3)	0.86 (t, 7.3)	
	Me	0.88 (t, 7.1)	0.86 (t, 7.3)	1.03 (t, 7.3)	1.03 (t, 7.1)	
	Fuc-1	4.77 (d, 7.4)	4.73 (d, 7.9)	4.79 (d, 6.8)	4.80 (d, 7.7)	4.73 (d, 7.9)
	2	4.19 (dd, 9.5,7.4)	4.16 (dd, 9.5, 7.9)	4.54 (dd, 9.5, 6.8)	4.56 (dd, 9.1, 7.7)	4.44 (dd, 9.4, 7.9)
	3	4.11 (dd, 9.5,3.4)	4.05 (dd, 9.5, 3.4)	4.19 (dd, 9.5, 3.3)	4.20 (dd, 9.1, 3.0)	4.11 (dd, 9.4, 3.4)
	4	4.02 (d, 3.4)	3.98 (d, 3.4)	3.93 (d, 3.3)	3.93 (d, 3.0)	3.92 (d, 3.4)
	5	3.80 (q, 6.3)	3.75 (q, 6.4)	3.82 (q, 6.8)	3.83 (q, 6.3)	3.75 (q, 6.4)
	6	1.50 (d, 6.3)	1.53 (d, 6.4)	1.52 (d, 6.8)	1.52 (d, 6.3)	1.50 (d, 6.4)
	Rha′-1	5.50 (d, 1.5)	5.50 (d, 1.2)	6.38 (d, 1.7)	6.40 (d, 1.4)	6.19 (d, 1.2)
	2	5.95 (dd, 2.2, 1.5)	5.94 (dd, 3.3, 1.2)	5.29 (dd, 3.3, 1.7)	5.27 (dd, 2.2, 1.4)	4.74 (dd, 3.3, 1.2)
	3	5.06 (dd, 9.6, 2.2)	5.01 (dd, 9.6, 3.3)	5.61 (dd, 9.4, 3.3)	5.70 (dd, 9.6, 2.2)	4.52 (dd, 9.6, 3.3)
	4	4.26 (dd, 9.6, 9.3)	4.23 (dd, 9.8, 9.6)	4.67 (dd, 9.7, 9.4)	4.73 (t, 9.6)	4.39 (dd, 9.8, 9.6)
	5	4.50 (dq, 9.3, 6.0)	4.49 (dq, 9.8, 5.8)	5.02 (dd, 9.7, 5.8)	5.10 (dd, 9.6, 6.2)	4.30 (dd, 9.8, 5.9)
	6	1.67 (d, 6.0)	1.70 (d, 5.8)	1.59 (d, 5.8)	1.60 (d, 6.2)	1.54 (d, 5.9)
	Rha″-1	6.23 (d, 1.6)	6.05 (d, 1.6)	5.63 (d, 1.7)	5.92 (d, 1.4)	6.21 (d, 1.6)
	2	4.99 (dd, 3.0, 1.6)	6.07 (dd, 3.2, 1.6)	5.83 (dd, 3.3, 1.7)	4.77 (dd, 3.0, 1.4)	4.62 (dd, 3.2, 1.6)
	3	5.85 (dd, 9.3, 3.0)	4.75 (dd, 9.0, 3.2)	4.64 (dd, 9.0, 3.3)	5.74 (dd, 9.3, 3.0)	4.58 (dd, 9.0, 3.2)
	4	4.62 (dd, 9.6, 9.3)	4.25 (dd, 9.4, 9.0)	4.27 (dd, 9.4, 9.0)	4.57 (dd, 9.6, 9.3)	4.29 (dd, 9.5, 9.0)
	5	4.46 (dq, 9.6, 6.2)	4.46 (dq, 9.4, 6.0)	4.38 (dq, 9.4, 6.0)	4.42 (dq, 9.6, 6.0)	4.31 (dq, 9.5, 6.0)
	6	1.67 (d, 6.2)	1.64 (d, 6.0)	1.65 (d, 6.0)	1.64 (d, 6.0)	1.55 (d, 6.0)
	Rha‴-1	5.70 (d, 1.6)	6.20 (brd, 1.1)	6.13 (d, 1.1)	5.59 (d, 1.4)	6.27 (d, 1.1)
	2	5.94 (dd, 3.2, 1.6)	6.23 (dd, 3.3, 1.1)	6.21 (dd, 3.3, 1.1)	5.85 (dd, 3.3, 1.4)	4.79 (dd, 3.3, 1.1)
	3	4.74 (dd, 9.9, 3.2)	4.80 (dd, 9.7, 3.3)	4.75 (dd, 9.6, 3.3)	4.63 (dd, 9.5, 3.3)	4.43 (dd, 9.6, 3.3)
	4	5.84 (t, 9.9)	5.87 (dd, 9.9, 9.7)	5.83 (dd, 9.8, 9.6)	5.80 (dd, 9.9, 9.5)	4.25 (dd, 9.9, 9.6)
	5	4.43 (dq, 9.9, 6.3)	4.53 (dq, 9.9, 5.8)	4.48 (dq, 9.8, 6.2)	4.36 (dq, 9.9, 6.3)	4.82 (dq, 9.9, 6.0)
	6	1.50 (d, 6.3)	1.58 (d, 5.8)	1.55 (d, 6.2)	1.52 (d, 6.3)	1.57 (d, 6.0)

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**5** (in pyridine-d_5, 150 MHz)

carbon	1	2	3	4	5
11	82.3	82.5	79.3	79.8	78.2
Cin-1	166.7	166.4	166.3	166.5	
2	118.5	118.7	118.6	118.5	
3	145.5	145.3	145.2	145.5	
4	134.6	134.8	134.7	134.6	
5,9	129.2	129.2	129.1	129.2	
6,8	128.6	128.6	128.5	128.6	
7	130.7	130.7	130.6	130.7	
C═O	173.2	173.1	173.2	173.1	176.1
C═O	173.7	173.2	173.6	173.6	
C═O	173.8	173.6	174.8	174.4	
Me	14.2	14.6	14.5	14.3	14.3
Me	14.3	14.6	14.6	14.3	
Me	14.3	14.6	14.9	14.6	
Fuc-1	104.4	104.5	101.6	101.7	101.4
2	80.2	80.2	73.2	73.0	75.1
3	73.4	73.7	76.8	76.9	76.6
4	73.0	73.1	73.6	73.6	73.4
5	70.9	70.8	71.3	71.2	71.2
6	17.4	17.7	17.2	17.8	17.4
Rha′-1	98.8	98.7	100.4	100.3	101.5
2	73.9	73.9	69.6	69.7	72.7
3	70.3	70.3	78.2	79.0	73.2
4	81.0	81.4	78.1	76.1	80.6
5	68.8	69.0	67.7	67.4	67.2
6	19.4	19.7	19.1	19.4	19.4
Rha″-1	103.4	100.4	100.3	102.4	102.9
2	70.0	74.2	74.1	70.3	73.1
3	75.6	71.1	70.6	75.2	73.4
4	79.1	80.4	80.5	79.1	79.6
5	68.6	68.4	68.3	69.0	68.4
6	18.8	19.2	18.7	18.5	18.9
Rha″-1	100.5	100.5	100.4	100.6	102.8
2	74.2	74.2	73.9	74.0	72.4
3	68.0	68.3	68.2	67.8	72.8
4	74.9	75.4	75.2	74.9	73.9
5	68.3	68.2	68.1	68.2	70.2
6	17.9	18.4	18.0	17.3	18.4

showed four ester carbonyl carbons at δ 173.8, 173.7, 173.2, and 166.4 and four anomeric carbons at δ 98.8, 100.5, 103.4, and 104.4 (Table 2). Detailed assignments of the proton and carbon signals of **1** were obtained using a group of NMR experiments (COSY, HMQC, HMBC, and ROESY). In a comparison of the ^{1}H NMR spectrum with that of **5**, the signals of H-2 (δ 5.95) of Rha′, H-2 (δ 5.85) of Rha″, and H-2 (δ 5.94) and H-4 (δ 5.84) of Rha‴ were shifted by +1.21, +1.27, +1.15, and +1.59 ppm, respectively. The HMBC spectrum of **1** permitted the unambiguous assignments of the esterified positions of the oligosaccharide core to be made from the correlations between the carbonyl ester group and the pyranose ring proton. The HMBC spectrum showed correlations between H-2 of Rha′ (δ 5.95)/C-1 of Jla (δ 173.2), H-3 of Rha″ (δ 5.85)/C-1 of Org (δ 173.7), H-2 of Rha‴ (δ 5.94)/the signal at δ 166.4 correlated with those of the olefinic protons due to the cinnamoyl, and H-4 of Rha‴ (δ 5.84)/C-1 of Org (δ 173.8), respectively. Therefore, these observations suggested that the carboxyl group of jalapinolic acid combined with C-2 of Rha′ to form a macrocyclic ring, with the cinmamoyl moiety attached at C-2 of Rha‴ and the dodecanoyl and decanoyl moieties located at C-3 of Rha″ and/or C-4 of Rha‴. In order to clarify the location of the ester linkage of the two organic acids, **1** was acylated to give a pentaacetate (**1a**). The positive FABMS of **1a** exhibited, besides a $[M + Na]^+$ ion peak at *m/z* 1538, a diagnostic fragment ion peak at *m/z* 501 [HRFABMS *m/z* 501.2901 (calcd for $C_{29}H_{41}O_7$, 501.2853)] assignable to a dodeca unit attached at C-4 of Rha‴, but no fragment ion peak at *m/z* 473, which would be expected if a deca unit had been located at this position. Accordingly, the structure of **1** was concluded to be (*S*)-jalapinolic acid 11-*O*-(2-*O*-*trans*-cinnamoyl)-[(4-*O*-*n*-dodecanoyl)]-α-L-rhamnopyranosyl-(1→4)-*O*-(3-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside, intramolecular 1,2″-ester.

Ipomotaoside B (**2**) exhibited a molecular formula of $C_{73}H_{120}O_{21}$ ($[M + Na]^+$, *m/z* 1355.8227), differing from **1** by an additional C_2H_4 unit. Also, the negative FABMS of **2** exhibited fragment ion peaks at *m/z* 1149 [M − 182 (dodeca) − H]$^-$, 1019 [1149 − 130 (cin)]$^-$, 837 [1019 − 182 (dodeca)]$^-$, 545, 417, and 271, suggesting that **2** consists of one unit of *trans*-cinnamic acid, two units of

n-dodecanoic acid, and **5**, with the jalapinolic acid unit esterified with the first rhamnose (Rha′). The sites of the four ester linkages in **2** were defined by use of the HMBC spectrum, and correlations were exhibited between H-2 of Rha′ (δ 5.94)/C-1 of Jla (δ 173.1), H-2 of Rha″ (δ 6.07)/C-1 of dodeca (δ 173.2), H-2 of Rha‴ (δ 6.23)/C-1 of cin (δ 166.4), and H-4 of Rha‴ (δ 5.87)/C-1 of dodeca (δ 173.6), respectively. Hence, the structure of ipomotaoside B (**2**) was concluded to be (*S*)-jalapinolic acid 11-*O*-(2-*O*-*trans*-cinnamoyl)-[(4-*O*-*n*-dodecanoyl)]-α-L-rhamnopyranosyl-(1→4)-*O*-(2-*O*-*n*-dodecanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside, intramolecular 1,2″-ester.

Ipomotaoside C (**3**) gave the same molecular formula, $C_{71}H_{116}O_{21}$ ($[M + Na]^+$, *m*/*z* 1327.7927), as **1**. The negative FABMS of **3** exhibited the same fragment peaks at *m*/*z* 1149 [M − 154 (deca) − H]$^-$, 1019 [1149 − 130 (cin)]$^-$, and 837 [1019 − 182 (dodeca)]$^-$, in addition to the same diagnostic fragment ion peaks at *m*/*z* 545, 417, and 271, suggesting it to be an isomer of **1**. In the HMBC spectrum of **3**, a macrocyclic ring was formed between jalapinolic acid and C-3 of Rha′ at δ 5.61 (dd, $J = 9.4, 3.3$ Hz), as shown by correlation between H-3 of Rha′/C-1 of Jla (δ 174.8), along with correlations with H-2 of Rha″ (δ 5.83)/C-1 of Org (δ 173.2), H-2 of Rha‴ (δ 6.21)/C-1 of cin (δ 166.4), and H-4 of Rha‴ (δ 5.83)/C-1 of Org (δ 173.6) similar to **2**. Using a similar strategy to that for **1**, acetylation of **3** gave a pentaacetate (**3a**), and its FABMS showed a diagnostic fragment ion at *m*/*z* 501 [HRFABMS *m*/*z* 501.2873 ($C_{29}H_{41}O_7$)] along with a $[M + Na]^+$ ion peak at *m*/*z* 1538, but no fragment ion peak at *m*/*z* 473. Consequently, the structure of ipomotaoside C (**3**) was concluded to be (*S*)-jalapinolic acid 11-*O*-(2-*O*-*trans*-cinnamoyl)-[(4-*O*-*n*-dodecanoyl)]-α-L-rhamnopyranosyl-(1→4)-*O*-(2-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside, intramolecular 1,3″-ester.

Ipomotaoside D (**4**) gave the same molecular formula of $C_{71}H_{116}O_{21}$ and fragment ion peaks at *m*/*z* 1173 [M − 130 (cin) − H]$^-$, 1019 [1173 − 154 (deca)]$^-$, 837 [1019 − 182 (dodeca)]$^-$, 545, 417, and 271 similar to those of **1** and **3** in the FABMS. The pentaacetate (**4a**) showed the same diagnostic fragment ion peak at *m*/*z* 501 [HRFABMS *m*/*z* 501.2859 ($C_{29}H_{41}O_7$)], but no peak at *m*/*z* 473. These MS data and the ^{1}H NMR spectra of **3** and **4** differed only in the position of the decanoyl residue. The HMBC spectrum of **4** gave the correlations of H-3 of Rha′ (δ 5.70)/C-1 of Jla (δ 174.4), H-3 of Rha″ (δ 5.74)/C-1 of deca (δ 173.1), H-2 of Rha‴ (δ 5.85)/C-1 of cin (δ 166.4), and H-4 of Rha‴(δ 5.80)/C-1 of deca (δ 173.6). These findings clarified that the deca at C-2 in **3** is transposed to C-3 in **4**. Thus, the structure of ipomotaoside D (**4**) was concluded to be (*S*)-jalapinolic acid 11-*O*-(2-*O*-*trans*-cinnamoyl)-[(4-*O*-*n*-dodecanoyl)]-α-L-rhamnopyranosyl-(1→4)-*O*-(3-*O*-*n*-decanoyl)-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-fucopyranoside, intramolecular 1,3″-ester.

The resin glycosides are known to exhibit several biological effects such as cytotoxicity to cancer cells and antimicrobial, antifungal,[13,14] antituberculosis,[15] and antidepressant activities.[16] However, anti-inflammatory activity had not been reported. In one of the inflammatory processes, two distinct isoforms of the cyclooxygenase enzymes, COX-1 and COX-2, convert arachidonic acid to prostaglandins. The inducible COX-2 enzyme is associated with inflammatory conditions, whereas extensively expressed COX-1 enzyme is responsible for the cytoprotective effects of prostaglandins.[17] The enzymes COX-1 and COX-2 have been used extensively as tools for studying the anti-inflammatory effects of natural compounds.[18−22] Each of the isolates from *I. batatas* was evaluated for its inhibitory activity against these enzymes, using a protocol according to Futaki et al.[23] Aspirin was used as positive control, and the results are listed in Table 3. Compound **1** showed an equivalent potency against both COX-1 and COX-2 to aspirin, whereas weak or no inhibitory activity was observed for compounds **2**−**4**. The degree to which these resin glycosides inhibited inflammatory mediators might depend on the size of the macrocyclic structure in each molecule. Our results suggest that a mixture of resin glycosides contained in the aerial parts of *I. batatas* is capable of inhibiting COX-1 and -2 enzymes. Therefore, their consumption may contribute to reduced inflammation.

Table 3. IC_{50} Values[a] (μM) of Compounds **1**−**4**

compound	COX-1	COX-2
1	9.3	14.5
2	132.0	147.0
3	NI[b]	NI[b]
4	NI[b]	NI[b]
aspirin	4.5	13.9

[a] IC_{50} based on triplicate five-point titration. [b] NI: no inhibition.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a JASCO DIP-1000 polarimeter. UV and IR spectra were recorded on a Shimazu UV-1650PC and a JASCO FT/IR-410 spectrophotometer, respectively. NMR spectra were measured on a Varian UNITY 600 spectrometer in C_5D_5N using TMS as internal standard. NMR experiments included COSY, DEPT, HMQC, HMBC, and ROESY. Coupling constants (*J* values) are given in Hz. MS were measured on a JEOL JMS-HX 100 mass spectrometer. Silica gel column chromatography was performed on Kieselgel 60 (230−400 mesh). HPLC was performed on a JASCO PU-1580 HPLC system equipped with a JASCO UV-970 detector.

Plant Material. *Ipomoea batatas* was collected at Nakagawa, Tokushima Prefecture, Japan, in October 2002. The plant was identified by one of the authors (S.A.), and a voucher specimen (TB 5427) was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. Dried aerial parts (2.0 kg) of *I. batatas* were extracted with EtOAc at room temperature for six weeks. The EtOAc extract (50 g) was subjected to silica gel column chromatography, eluted with hexane−EtOAc (10:1 → 1:30) for elution to afford fractions 1−7. Fraction 5 (7.0 g) was passaged over a silica gel column, with hexane−EtOAc (3:7−0:10) for elution, to afford fractions 5-1−4. Fraction 5-2 was subjected to silica gel column chromatography, eluted with hexane−EtOAc (3:7−0:10), and was purified finally by preparative HPLC (ODS, 88% MeOH), to afford ipomotaosides A (**1**, 35.0 mg), B (**2**, 90.0 mg), and C (**3**, 75.0 mg). Fraction 5-3 was purified by preparative HPLC (ODS, 80−100% MeOH) to afford ipomotaosides A (**1**, 521.5 mg), B (**2**, 20.0 mg), and D (**4**, 195.0 mg).

Ipomotaoside A (1): white powder; mp 100−102 °C; $[\alpha]^{25}_D$ −27.9 (*c* 1.5, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.98), 237 (4.03), 244 (3.88) nm; FT-IR (dry film) ν_{max} 3445, 1715, 1635, 1050 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 1327.7911 (calcd for $C_{71}H_{116}O_{21}Na$, 1327.7907).

Ipomotaoside B (2): white powder; mp 112−114 °C; $[\alpha]^{25}_D$ −8.9 (*c* 1.7, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.06), 238 (4.24), 246 (3.87) nm; FT-IR (film) ν_{max} 3450, 1740, 1640, 1075 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 1355.8227 (calcd for $C_{73}H_{120}O_{21}Na$, 1355.8220).

Ipomotaoside C (3): white powder; mp 105−107 °C; $[\alpha]^{25}_D$ −37.4 (*c* 3.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.98), 237 (4.03), 244 (3.88) nm; FT-IR (film) ν_{max} 3440, 1730, 1640, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 1327.7927 (calcd for $C_{71}H_{116}O_{21}Na$, 1327.7907).

Ipomotaoside D (4): white powder; mp 106−108 °C; $[\alpha]^{25}_D$ −18.3 (*c* 3.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.98), 237 (4.03), 244 (3.88) nm; FT-IR (dry film) ν_{max} 3445, 1740, 1640, 1050 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 1327.7962 (calcd for $C_{71}H_{116}O_{21}Na$, 1327.7907).

Alkaline Hydrolysis of Compounds 1−4. A mixture of **1**−**4** (70.0 mg) in MeOH (2 mL) was treated with 5% KOH (2 mL), and the mixture was heated at 90 °C for 2 h. The reaction mixture was adjusted to pH 4.0 with 5% HCl and extracted with diethyl ether to give a mixture of organic acids. A solution of the organic acids (8 mg) and *O*-*p*-nitrobenzyl-*N*,*N*′-diisopropylisourea (30 mg) in CH_2Cl_2 (3 mL) was heated at 80 °C for 2 h. The reaction mixture was evaporated under reduced pressure to afford their *p*-nitrobenzyl esters. The *p*-nitrobenzyl esters were compared by HPLC (C_{30}, 90% MeOH, 1 mL/min, 273 nm)

with authentic samples. The organic acid portion gave the following peaks, t_R: *trans*-cinnamic acid, 5.2 min [HREIMS *m/z* 283.0852 (calcd for $C_{16}H_{13}O_4N$, 283.0845)]; *n*-decanoic acid, 11.6 min [HREIMS *m/z* 307.1785 (calcd for $C_{17}H_{25}O_4N$, 307.1784)]; *n*-dodecanoic acid, 21.0 min [HREIMS *m/z* 335.2102 (calcd for $C_{19}H_{29}O_4N$, 335.2097)]. The *n*-BuOH layer was subjected to silica gel column chromatography, eluting with CH_2C1_2−MeOH−H_2O (8:3:1), to afford operculinic acid C (**5**, 37 mg).[11] Compound **5**: amorphous solid; $[\alpha]^{25}_D$ −65.3 (*c* 1.7, MeOH); FT-IR (dry film) ν_{max} 3410 (OH), 1700 (C=O) cm^{-1}; ^1H and ^{13}C NMR (C_5D_5N, 600 MHz), see Tables 1 and 2; FABMS *m/z* 837 $[M - H]^-$.

Acid Hydrolysis of Compound 5. A solution of **5** (30 mg) in 5% H_2SO_4−dioxane was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and then was adjusted to pH 4.0 with 5% HCl and extracted with diethyl ether, and the organic layer was treated with diazomethane and then purified over silica gel (hexane−EtOAc, 3:7) to furnish methyl jalapinolate[11] (**6**, 6 mg). Compound **6**: $[\alpha]^{25}_D$ −0.3 (*c* 0.7, MeOH); HREIMS *m/z* 286.2533 (calcd for $C_{17}H_{34}O_3$, 286.2508).

The H_2O layer was neutralized with Amberlite IRA-35 and evaporated in vacuo to dryness. The identification and the D or L configuration of each sugar was determined by using RI detection (Shimadzu RID-10A) and chiral detection (Shodex OR-1) by HPLC (Shodex RSpak NH2P-50 4D, CH_3CN−H_2O−H_3PO_4, 95:5:1, 1 mL/min, 47 °C), by comparison with an authentic sugar (10 mmol each of D-fuc and L-rha). The sugar portion gave the following peaks: L-(+)-rha 6.40 min; D-(+)-fuc 8.10 min.

Acetylation of Compounds 1, 3, and 4. Each compound (2−3 mg) was acetylated with Ac_2O−pyridine (1:1) to give the acetates **1a**, **3a**, and **4a**. **1a**: (+)-FABMS *m/z* 1538 (16), 501 (39), 131 (100); HRFABMS *m/z* 1537.8424 (calcd for $C_{81}H_{126}O_{26}Na$, 1537.8435) and *m/z* 501.2901 (calcd for $C_{29}H_{41}O_7$, 501.2853). **3a**: (+)-FABMS *m/z* 1538 (18), 501 (26), 131 (100); HRFABMS *m/z* 1537.8448 (calcd for $C_{81}H_{126}O_{26}Na$, 1537.8435) and *m/z* 501.2873 (calcd for $C_{29}H_{41}O_7$, 501.2853). **4a**: (+)-FABMS *m/z* 1538 (18), 501 (39), 131 (100); HRFABMS *m/z* 1537.8451 (calcd for $C_{81}H_{126}O_{26}Na$, 1537.8435) and *m/z* 501.2859 (calcd for $C_{29}H_{41}O_7$, 501.2853).

COX-1- and COX-2-Catalyzed Prostaglandin Biosynthesis Assay in Vitro. Experiments were performed according to Futaki et al.,[23] with minor modification. In brief, two units of COX-1/COX-2 enzyme were suspended in 0.1 M of Tris-HCl buffer (pH 7.5) containing hematin (1 mM) and phenol (2 mM), as cofactors. The reaction medium was preincubated with sample for 2 min at 37 °C, and 51.4 μM [1-^{14}C] arachidonic acid (Sigma, St. Louis, MO) was added. The reaction mixture was incubated for 2 min at 37 °C. To terminate the reaction and extract PGE_2, 400 μL of *n*-hexane−EtOAc (2:1) was added to the reaction mixture, and the preparation was centrifuged at 2000 rpm for 1 min. The organic solvent phase was discarded. The extraction procedure was repeated twice; then 50 μL of EtOH was added to the aqueous phase and the preparation centrifuged at 2000 rpm for 1 min. The amount of PGE_2 was measured by radioimmunoassay using a liquid scintillation counter. COX-1 (EC1.14.99.1, isolated from ram seminal vesicles, Cayman Chemical Company, Ann Arbor, MI) and COX-2 (isolated from sheep placenta, purity 70%, Cayman Chemical Company) were used.

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Supporting Information Available: 1D and 2D NMR spectra of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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