Novel Trisaccharide Fatty Acid Ester Identified from the Fruits of *Morinda citrifolia* (Noni)

Mingfu Wang,[†] Hiroe Kikuzaki,[‡] Katalin Csiszar,[§] Charles D. Boyd,[§] Alika Maunakea,[§] Sheri F. T. Fong,[§] Geetha Ghai,[†] Robert T. Rosen,[†] Nobuji Nakatani,[‡] and Chi-Tang Ho*[,†]

Department of Food Science and Center for Advanced Food Technology, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901-8520; Department of Food and Nutrition, Osaka City University, 3-3-138 Sugimoto Sumiyoshi, Osaka, Japan; and Pacific Biomedical Research Center, University of Hawaii, 1993 East-West Road, Honolulu, Hawaii 96822

Two known glycosides and a novel trisaccharide fatty acid ester were isolated from the *n*-butanol-soluble fraction of the fruits of *Morinda citrifolia* (noni). Structure determination was carried out by spectral techniques such as MS, IR, NMR, and 2D-NMR. The novel trisaccharide fatty acid ester was elucidated as 2,6-di-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-glucopyranose. The known compounds were identified as rutin and asperulosidic acid.

Keywords: *Noni; Morinda citrifolia; trisaccharide fatty acid ester; 2,6-di-O-(β-D-glucopyranosyl-1-O-octanoyl-β-D-glucopyranose; rutin; asperulosidic acid*

INTRODUCTION

Morinda citrifolia (Rubiaceae), commonly known as noni, is a plant typically found in the Hawaiian and Tahitian islands. It is believed to be one of the most important plants brought to Hawaii by the first Polynesians (Levand and Larson, 1979). The plant is a small evergreen tree growing in the open coastal regions. The fruit can grow to a size of 12 cm and results from coalescence of the inferior ovaries of many closely packed flowers. It has a foul taste and a soapy smell when mature. The bark, stem, root, leaf, and fruit have been used traditionally as a folk remedy for many diseases including diabetes, hypertension, and cancer (Hirazumi et al., 1994, 1996). The fruits of this plant were also used as foods in time of famine, whereas the roots were used to produce a yellow or red dye for cloth. In earlier studies, ricinoleic acid was found in the seeds (Daulatabad et al., 1989), whereas anthraquinones, including morenone 1, morenone 2, and 7-hydroxy-8-methoxy-2-methylanthraquinone, have been identified in the root of noni (Rusia and Srivastava, 1989; Jain and Srivastava, 1992). Anthraquinone glycosides have been isolated from the heartwood of *Morinda citrifolia* (Srivastava and Singh, 1993). Studies on the chemical components of the flowers of noni have resulted in the identification of several anthraquinone glycosides and flavone glycosides (Tiwari and Singh, 1977; Singh and Tiwari, 1976), whereas *β*-sitosterol and ursolic acid have been isolated from the leaves (Ahamad and Bano, 1980). Although the fruits of noni have been used as a food, very few reports on the chemical components of the fruits are available (Levand and Larson, 1979). Several compounds including acetyl derivatives of asperuloside, glucose, caproic acid, and caprylic acid have been identified in fruits (Levand and Larson, 1979).

The juice of noni fruits has been shown to prolong the life span of mice implanted with Lewis lung carcinoma (Hirazumi et al., 1994). It was proposed that the fruits of noni might suppress the growth of tumors by stimulating the immune system (Hirazumi et al., 1994). Later, the same authors provided evidence supporting their hypothesis and partly identified a polysaccharide as an active component (Hirazumi et al., 1996). Individual compounds from noni have also been tested for antitumor activity. Damnacanthal, an anthraquinone isolated from the chloroform extract of the roots of noni, has been found to be a new inhibitor of *ras* function and to help to suppress the activated *ras*-expressing tumors. The extracts of noni roots have also been found to possess a significant, dose-dependent, central analgesic activity in the treated mice (Younos et al., 1990).

Because limited reports on the fruits of noni were available in the literature, it was thought to be desirable to carry out systematic chemical investigations on the fruits of this plant. The present studies purports to carry out the chemical investigation on the fruits of this plant. The isolation and structural elucidation of a novel trisaccharide fatty acid ester together with two known glycosides are being reported.

MATERIALS AND METHODS

Chemicals. Silica gel (130−270 mesh), Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO), and a Lichroprep RP-18 column were used for column chromatography. All solvents used for chromatographic isolation were of analytical grade.

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a VXR-200 instrument (Varian Inc., Palo Alto, CA), operating at 200 and 50 MHz, or on a U-500 instrument (Varian Inc.), operating at 500 and 125 MHz, respectively. Compounds were analyzed in CD_3OD and DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC were performed on a U-500 instrument (Varian Inc.). FAB mass spectra were recorded on a Finnigan MAT-90 instrument. APCI MS was obtained on a Fisons/VG Platform II mass spectrometer. FT-

* Corresponding author [fax (732) 932-8004; e-mail ho@aesop.rutgers.edu].
[†] Rutgers University.
[‡] Osaka City University.
[§] University of Hawaii.

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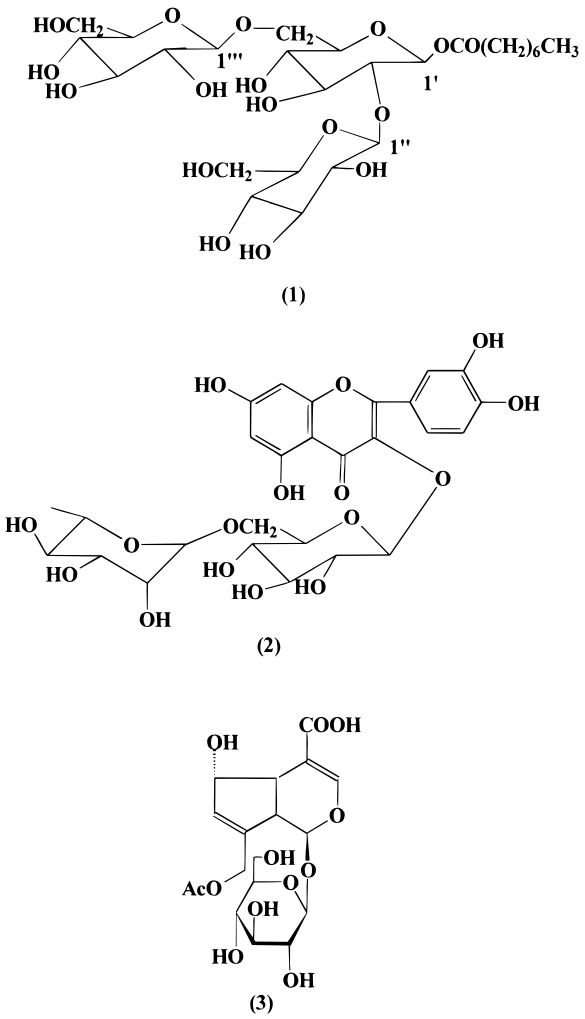

Figure 1. Structures of compounds identified in the fruits of noni: (1) 2,6-di-*O*-(*β*-D-glucopyranosyl-1-*O*-octanoyl-*β*-D-glucopyranose; (2) rutin; (3) asperulosidic acid.

IR spectra were obtained with a Perkin-Elmer 1600 apparatus. Thin-layer chromatography was performed on Sigma-Aldrich TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution.

Extraction and Isolation Procedure. Noni fruits were freeze-dried. The dried noni fruit (200 g) pieces were extracted with 95% ethanol (1 L) at room temperature for 1 week. The extract was concentrated to dryness under reduced pressure, and the residue was suspended in water (500 mL) and partitioned successively with hexane (3 × 500 mL), ethyl acetate (3 × 500 mL), and *n*-butanol (3 × 500 mL). The *n*-butanol-soluble fraction (40 g) was subjected to column chromatography on 500 g of silica gel and eluted with ethyl acetate/methanol/water as eluent with increasing methanol and water content (10:1:1, 6:1:1, 6:1:1, 4:1:1, 3:1:1, 2:1:0.5, each 1000 mL), and 500 mL fractions were collected. Fractions 9 and 10 were combined together and rechromatographed on a Lichroprep RP-18 column using methanol/water (1:3, 1000 mL; 2:3, 1000 mL) and methanol to obtain pure compound **1** (1 g) (Figure 1). Fraction 4 was subjected to a Sephadex LH-20 column (eluted with methanol) to get two fractions; the first fraction was then subjected to a Lichroprep RP-18 column eluted with methanol/water (1:3, 1000 mL, 2:3, 1000 mL) to give five subfractions, and then subfraction 1 was subjected to a silica gel column using ethyl acetate/methanol/water (10:1:1) as eluent to obtain 100 mg of compound **2**. The second fraction was subjected to a silica gel column using ethyl acetate/methanol/water (7:1:1) to yield 100 mg of compound **3**.

RESULTS AND DISCUSSION

The *n*-butanol-soluble fraction obtained from the ethanol extracts of the fruits of *M. citrifolia* was subjected to repeated chromatography on silica gel, Sephadex LH-20, and RP-10 Lobar columns to obtain three major compounds. The structures of these three compounds were determined by spectral methods. A novel compound **1** was obtained as a white powder. The IR (KBr) spectrum showed absorption at ν 3502 (O—H), 2918 (C—H), 1741 (C═O), 1382 (C—H), 1146 (C—O), and 1060 (C—O). The positive ion FAB-MS exhibited a peak at *m/z* 653 $[M + Na]^+$, compatible with the molecular formula $C_{26}H_{46}O_{17}$. The ^{1}H NMR spectrum of **1** displayed the anomeric proton signals of the monosaccharides at δ 5.59 (1H, d, J = 7.8 Hz, for glucose), 4.55 (1H, d, J = 7.8 Hz, for glucose), and 4.31 (1H, d, J = 7.8 Hz, for glucose), which led to the assignment of the anomeric configuration of the monosaccharides as β (Du et al., 1998). In addition, the signals at δ 0.90 (3H, t, J = 7.1 Hz), 1.30−1.35 (8H, m), 1.62 (2H, m), 2.38 (1H, dt, J = 16.6, 7.3 Hz), and 2.47 (1H, dt, J = 16.6, 7.3 Hz) suggested the possible presence of an octanoic acid (caprylic acid) moiety in this molecule. The ^{13}C NMR spectrum of **1** supported our hypothesis that one octanoic acid ester exists in this compound by signals at δ 14.5 (q), 23.7 (t), 25.6 (t), 30.1 (t), 30.2 (t), 32.9 (t), 34.9 (t), and 174.0 (s). The ^{13}C NMR also displayed signals at δ 62.7 (t, 2C), 71.4 (d), 71.5 (d), 75.1 (d), 76.0 (d), 77.7 (d), 78.0 (d, 2C), 78.2 (d), 104.6 (d), and 105.6 (d), attributable to C-1 and C-6 of two terminal glucose units, respectively, whereas the signals observed at δ 69.4 (t), 70.6 (d), 77.6 (d, 2C), 82.4 (d), and 94.0 (d) were due to the inner glucose. The glycosylation shifts of the C-2 and C-6 signals by comparison with the signals of methyl β-D-glucopyranoside suggested that the two terminal glucose units were possibly connected to C-2 and C-6 of the inner glucose (Wang and Yu, 1998).

Analysis of the COSY, NOESY, HMQC, and HMBC spectra led to the complete assignment of the ^{1}H and ^{13}C signals (Table 1). The linkage of the three sugars and the fatty acid moiety was deduced and proved by the HMBC experiments, in which correlation contours were observed between H-1 of the inner glucose (δ 5.57, 1H, d, J = 7.8 Hz) and the carbonyl group of octanoic acid at δ 174.0 ppm, between H-1 of one terminal glucose (δ 4.55, 1H, d, J = 7.8 Hz) and C-2 of the inner glucose at δ 82.4 ppm, and between H-1 of another terminal glucose (δ 4.31, 1H, d, J = 7.8 Hz) and C-6 of the inner glucose (δ 69.4 ppm). The above evidence resulted in the establishment of both the linkage sites and sequences of the sugar units as 2,6-di-*O*-(*β*-D-glucopyranosyl-1-*O*-octanoyl-*β*-D-glucopyranose. This is further supported by the NOESY spectrum, in which the H-1 of one terminal glucose (δ 4.55, 1H, d, J = 7.8 Hz) showed significant correlation with H-2 of the inner glucose at δ 3.60 (1H, dd, J = 8.8, 7.8 Hz), whereas the H-1 of another terminal glucose at 4.31 ppm (1H, d, J = 7.8 Hz) had correlation with the H-6 of the inner glucose at δ 3.76 (1H, dd, J = 4.9, 11.5 Hz) and 4.14 (1H, dd, J = 11.5, 2.0 Hz).

The second compound was isolated as a yellow powder, the negative APCI MS exhibited a pseudomolecular ion peak at *m/z* 609 $[M - 1]^-$, and the positive APCI MS showed a significant pseudomolecualr ion peak at 611 $[M + 1]^+$, which together with the ^{1}H and ^{13}C NMR indicated a molecular formula of $C_{27}H_{30}O_{16}$

Table 1. ^{1}H and ^{13}C NMR Data of Compound 1 in CD_3OD

carbon	^{1}H NMR	^{13}C NMR
1		174.0 (s)
2	2.38 (1H, dt, J = 16.6, 7.3 Hz; H-2a)	34.9 (t)
	2.47 (1H,dt, J = 16.6, 7.3 Hz; H-2b)	
3	1.62 (2H, quint, J = 7.3 Hz)	25.55 (t)
4	1.30−1.35 (2H, m)	30.15 (t)
5	1.30−1.35 (2H, m)	30.13 (t)
6	1.30−1.35 (2H, m)	32.88 (t)
7	1.30−1.35 (2H, m)	23.7 (t)
8	0.90 (3H, t, J = 7.1 Hz)	14.5 (q)
1′	5.59 (1H, d, J = 7.8 Hz)	94.0 (d)
2′	3.60 (1H, dd, J = 8.8, 7.8 Hz)	82.4 (d)
3′	3.61 (1H, dd, J = 8.8, 8.6 Hz)	77.6 (d)
4′	3.48 (1H, dd, J = 9.8, 8.6 Hz)	70.6 (d)
5′	3.55 (1H, ddd, J = 9.8, 4.9, 2.0 Hz)	77.6 (d)
6′	3.76 (1H, dd, J = 11.5, 4.9 Hz)	69.4 (t)
	4.14 (1H, dd, J = 11.5, 2.0 Hz)	
1″	4.55 (1H, d, J = 7.8 Hz)	105.6 (d)
2″	3.18 (1H, dd, J = 9.3, 78 Hz)	76.0 (d)
3″	3.34 (1H, dd, J = 9.3, 9.3 Hz)	77.7 (d)
4″	3.26 (1H, dd, J = 9.3, 8.1 Hz)	71.4 (d)
5″	3.27 (1H, m)	78.0 (d)
6″	3.67 (1H,dd, J = 12.0, 5.0 Hz)	62.7 (d)
	3.82 (1H, dd, J = 12.0, 2.0 Hz)	
1‴	4.31 (1H, d, J = 7.8 Hz)	104.6 (d)
2‴	3.20 (1H, dd, J = 9.3, 7.8 Hz)	75.1 (d)
3‴	3.36 (1H, dd, J = 9.3, 9.3 Hz)	78.2 (d)
4‴	3.27 (1H, dd, J = 9.3, 8.1 Hz)	71.5 (d)
5‴	3.23 (1H, m)	78.0 (d)
6‴	3.65 (1H, dd, J = 12,0, 5.4 Hz)	62.7 (t)
	3.85 (1H, dd. J = 12.0, 2.0 Hz)	

for compound **2**. The ^{1}H NMR showed signals at δ 12.61 (1H, s, 5-OH), 7.56 (2H, m, H-2′,6′), 6.86 (1H, d, J = 9.0 Hz, H-5′), 6.41 (1H, br s, H-8), 6.22 (1H, br s, H-6), 5.35 (1H, d, J = 7.4 Hz, H-glu-1), 4.40 (1H, H-rha-1), and 3.00−3.84 (sugar protons), 1.00 (3H, d, J = 6.0 Hz, H-rha-6). The ^{13}C NMR (50 MHz, in DMSO-d_6) showed data at δ 177.6 (s, C-4), 164.3 (s, C-7), 161.5 (s, C-5), 156.9 (s, C-9), 156.7 (s, C-2), 148.7 (s, C-4′), 145.0 (s, C-3′), 133.5 (s, C-3), 121.9 (s, C-1′), 121.4 (d, C-6′), 116.5 (d, C-5′), 115.5 (d, C-2′), 104.2 (s, C-10), 101.4 (d, C-glc-1), 101.0 (d, C-rha-1), 98.9 (d, C-6), 93.9 (d, C-8), 76.7 (d, C-glc-3), 76.1 (d, C-glc-5), 74.3 (d, C-glc-2), 72.1 (d, C-glc-4), 70.8 (d, C-rha-3), 70.6 (d, C-rha-2), 70.3 (d, C-glc-4), 68.5 (d, C-rha-5), 67.3 (t, C-glc-6), and 18.0 (q, C-rha-6). These data were found to be identical with those of rutin (Agrawal et al., 1989). The presence of rutin in noni has been reported for the first time by us.

The third compound was isolated as a colorless oil. Its molecular formula, $C_{18}H_{24}O_{12}$, was deduced from negative APCI MS, which showed a pseudomolecular ion peak at m/z 431 $[M - 1]^-$, the positive APCI MS, which exhibited a significant pseudomolecular ion at m/z 450 $[M + NH_4]^+$, and the ^{1}H and ^{13}C NMR. The ^{1}H NMR spectrum showed signals at δ 7.66 (1H, br s, H-3), 6.03 (1H, br s, H-7), 5.08 (1H, d, J = 9.0 Hz, H-1), 4.73 (1H, d, J = 7.6 Hz, H-1′), 3.87 (1H, d, J = 11.4 Hz, H-6′), 3.20−3.72 (m, H-2′,3′,4′,5′,6′), 3.03 (1H, m, H-5), and 2.63 (1H, t, J = 8.3 Hz, H-9). ^{13}C NMR showed signals at δ 173.2 (s, C═O), 171.2 (s, C═O), 155.6 (d, C-3), 146.2 (s, C-8), 132.2 (d, C-7), 101.3 (d, C-1′), 100.9 (d, C-1), 78.9 (d, C-5′), 78.2 (d, C-3′), 75.7 (d, C-6), 75.2 (d, C-2′), 71.9 (d, C-4′), 64.1 (t, C-10), 63.3 (t, C-6′), 46.6 (d, C-9), 42.8 (d, C-5), and 21.1 (q, $COCH_3$). These spectral data are identical with those of asperulosidic acid (El-Nagger and Beal, 1980; Peng et al., 1998). Although earlier researchers have found this compound from several plants including a Chinese variety of noni, *Morinda officinalis* (Yoshikawa et al., 1995), this is the first time the isolation of asperulosidic acid from the fruits of noni, *Morinda citrifolia*, has been reported. Earlier pharmacological studies revealed that the compound had anticlastogenic and antimutagenicity activities (Nakamura et al., 1997).

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