Zanthosides A–D: Four Aromatic Glucosides from the Leaves of Zanthoxylum ailanthoides

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Introduction

Rutaceous plants growing in the Okinawa Islands were inhibited from immigrating into the mainland of Japan until early 1980, due to the spread of the Oriental fruit fly, Bactrocera dorsalis. A mated female B. dorsalis punctures the skin of mature fruit, e.g., Hirami lemon, Citrus despressa, and deposits eggs underneath a fruit’s skin. Eggs hatch to larvae and molt twice while feeding on the flesh of the fruit. In 1982, B. dorsalis was exterminated from the Okinawa Archipelago using a pheromone, methyl eugenol. It attracted male B. dorsalis and the collected males were extinguished. After that, immigration rules were eased. However, some of the Rutaceous plants, for example Murraya paniculate and M. koengii etc., are still under the regulation. The genus Zanthoxylum belonging to the family Rutaceae comprises about 200 species and is found in tropical and moderate areas of both hemispheres. Z. piperitum De Candolle is a well-known medicinal plant, and its seed-depleted matured husks are used for an aromatic and pungent stomachic, anthemlinthic, and so on. Young leaves and husks are also used for food seasonings. Z. ailanthoides Siebold et Zuccarini is a tall deciduous tree that grows in Japan (Honshu, Kyushu and Okinawa Islands), Taiwan, China, and the Philippines. In a previous paper, the isolation of an aliphatic glucoside, zanthoionic acid, and megastigmane glucosides named zanthoionosides A–E from the leaves of Z. ailanthoides collected in the Okinawa Island, was reported. From further investigations of the same plant, one coumarin derivative (1, 4.7 mg), two prenylated phenylpropanoids (2, 42.6 mg) and (3, 9.8 mg) and a phenethyl alcohol glucoside (4, 21.1 mg), termed zanthosides A–D (Fig. 1), were isolated along with known glucosides, (Z)-6-(4-B-p-glucopyranosyloxy-3-methylbut-2-en-1-yl)-7-hydroxycoumarin (5), skimmian (6), aesculin (7), thermesmin (8), nodakenin (9), decurcinol O-B-p-glucopyranoside (10), and picraquassioside B (11). This paper deals with the structural elucidation of the new compounds.

Results and Discussion

Four new compounds and seven known ones were obtained by extensive separation of the MeOH extract of the leaves of Z. ailanthoides using Diaion HP-20 column chromatography (CC), normal and reversed-phase silica gel CC, droplet counter-current chromatography (DCCC), and HPLC. The structures of new compounds were established mainly by NMR spectroscopic evidence (one- and two-dimensional spectroscopy), and known compounds were identified by comparison with data reported in the literature.

Zanthoside A (1), [α] 23D = −84.7, was isolated as an amorphous powder, and its elemental composition was determined to be C25H32O10 by high-resolution (HR) electrospray ionization (ESI) MS. The IR spectrum exhibited absorption bands ascribable to hydroxy groups (3308 cm −1 ), a carbonyl group (1734 cm −1 ), and an aromatic ring (1629 and 1573 cm −1 ). The presence of the aromatic ring was also supported by a UV absorption band at 320 nm. In the 1H-NMR spectrum, signals assignable to two singlet methyls, two aromatic protons, two olefinic protons in cis geometry [δ H 6.23 (d, J = 9.5 Hz) and 7.88 (d, J = 9.5 Hz)], two oxymethines, and an anomic proton [δ H 4.73 (J = 7.8 Hz)] were observed (Table 1). The 13C-NMR spectrum displayed 20 signals (Table 2). Six of these were typical of a hexopyranose and this sugar was found to be D-glucose by HPLC analysis of the hydrolyzate of 1 using a chiral detector. The remaining 14 signals comprised two methyls, two oxymethines, one oxygenated aliphatic tertiary carbon, eight sp 2 signals and a carbonyl carbon. The eight sp 2 signals expected to form an aromatic ring, included four substituted ones and two with aromatic protons and a disubstituted double bond. One of the aromatic protons was coupled with an oxymethine proton, and the other appeared as a singlet. This evidence suggested that the benzene ring was substituted at the 1, 2, 4, and 5 positions, and the degrees of unsaturation acquired by MS analysis suggested two rings other than the aromatic one and a sugar moiety were present in the scaffold. The crucial heteronuclear multiple-bond correlations (HMBC) between one of the olefinic protons at H-4 (δ H 7.88) and C-2
and C-5 (δH 163.3 and 129.4, respectively) and the ester absorption in the IR spectrum established the structure of 1 to be a coumarin derivative. Further HMBC correlation of H-5 (δH 7.70) and C-1/uni2032 (δC 69.3) suggested that the remaining five carbon unit was attached at the C-6 position, and this unit was also expected to have a cyclic system from the degree of unsaturation (Fig. 1). The 1H–1H correlation spectroscopy (COSY) correlation from H-1/uni2032 to H-2/uni2032, and HMBC correlations between H-1/uni2032 and C-3/uni2032 and H-2/uni2032 and C-4/uni2032 substantiated the planar structure of the aglycone of 1 to be decursidinol as shown Fig. 1. The position of the sugar linkage was determined to be on the hydroxy group at the 2/uni2033-position by HMBC correlation between H-1/uni2033 and C-2/uni2032, and the mode of linkage β from the coupling constant of the anomeric proton. Closely

Table 1. 1H-NMR Spectroscopic Data for Zanthosides A–D (1–4) (600 MHz, CD3OD)

|   | 1   | 2   | 3   | 4   |
|---|-----|-----|-----|-----|
| 1 | 27.3| 27.1| 28.2| 25.9|
| 2 | 114.5| 114.4| 123.6| 129.9|
| 3 | 163.3| 163.3| 157.4| 130.2|
| 4 | 145.8| 145.8| 153.9| 158.5|
| 5 | 129.4| 129.6| 117.2| 116.1|
| 6 | 123.8| 124.4| 128.5| 128.5|
| 7 | 157.4| 157.7| 26.4| 145.8|
| 8 | 105.3| 104.6| 35.8| 117.8|
| 9 | 156.2| 156.2| 177.8| 171.1|
| 10| 114.5| 114.4| 123.6| 129.9|

Table 2. 13C-NMR Spectroscopic Data for Zanthosides A–D (1–4) and trans-Decursidinol (1a) (150 MHz, CD3OD)

|   | 1   | 1a  | 2   | 3   | 4   |
|---|-----|-----|-----|-----|-----|
| 1 | 123.6| 129.9| 130.2| 119.6| 146.5|
| 2 | 113.6| 113.6| 104.8| 132.9| 146.8|
| 3 | 145.8| 145.8| 153.9| 158.5| 146.8|
| 4 | 129.4| 129.6| 117.2| 116.1| 117.0|
| 5 | 123.8| 124.4| 128.5| 128.5| 125.4|
| 6 | 157.4| 157.7| 26.4| 145.8| 39.6|
| 7 | 105.3| 104.6| 35.8| 117.8| 64.3|
| 8 | 156.2| 156.2| 177.8| 171.1| 104.4|
| 9 | 114.5| 114.4| 123.6| 129.9| 74.9|
| 10| 80.8| 81.5| 77.4| 133.8| 75.5|
| 11| 21.0| 19.8| 28.3| 18.0| 71.8|
| 12| 27.3| 27.1| 28.2| 25.9| 75.8|
| 13| 104.6| 102.6| 102.2| 126.7| 64.8|
| 14| 75.9| 75.0| 75.0| 107.1| 64.8|
| 15| 78.3| 78.3| 78.3| 149.5| 147.5|
| 16| 71.7| 71.4| 71.4| 139.8| 115.6|
| 17| 78.2| 78.2| 78.2| 149.5| 169.0|
| 18| 62.9| 62.6| 62.2| 107.1| 57.0|
| 19| 52.1| 57.0| 52.1| 57.0| 57.0|

m: multiplets or overlapped signals.
Therefore, the structure of 1 was elucidated to be (1 + (coupling constant between H-1 including the trans correlations of H-2 and H-8, and H-1 powder, and its elemental composition was determined to be ¼. The HMBC cross peaks between H-6 and C-7 and C-1 were observed. The position and mode of sugar linkage were determined to be at the hydroxy group in the 4-position by the HMBC spectrum and also by the coupling constant of the anomeric proton. The structure of 3 is shown in Fig. 1.

Zanthoside D (4), [α]25° +39.7, was isolated as an amorphous powder. Its elemental composition was determined to be C25H30O12 by HR-ESI-MS. The IR spectrum indicated the presence of a carbonyl and an aromatic ring similar to that of 3. In the 1H-NMR spectrum, there were two olefinic protons in a trans double bond, three aromatic protons, one singlet aromatic signal for two protons, four aliphatic protons for two contiguous methylenes, one methoxy signal for six protons, and an anomeric proton. The 13C-NMR spectrum displayed 22 signals, 12 of these were assigned to hexose and a trisubstituted aromatic ring. The hexose was identified with d-glucose by HPLC analysis of its hydrolysate, and the remaining ten signals consisted of 13 carbons, namely the presence of a symmetrically substituted aromatic ring was suggested. The HMBC correlations from methylene protons (H-2) to three symmetrically substituted aromatic ring was suggested. The symmetrical acyl group with two methoxy groups was expected to be sinapic acid from HMBC correlation between H-2 [δH 7.33 (d, J = 1.81 Hz) and C-7, H-7 and C-9, and H-2 with C-4 suggested one of the aromatic rings carried an ethanol side chain and from those H-2 and C-6 and C-4, and H-5 and H-6 with C-3 and C-2, respectively, 1,3,4-substitution of this aromatic ring was established (Fig. 3). Further HMBC correlation between H-1 and C-3 confirmed the position of the sugar linkage and the mode of the linkage was 3β. The symmetrical acyl group with two methoxy groups was expected to be sinapic acid from HMBC correlation between H-7 [δH 7.64] and C-2 (δC 107.1) (Fig. 3), and the position of the ester linkage was at the hydroxyl group at C-6 from HMBC correlation and downfield shifts of the H-6 proton in the 1H-NMR spectrum. Therefore, the structure of 4 was elucidated to be 3,4-dihydroxyphenethyl alcohol 3-O-β-d-glucopyranoside 6-O-synapate, as shown in Fig. 1.

The structure of zanthoside A (1) was unambiguously determined to be a glucoside of prenylated coumarin, including the absolute stereochemistry of the aglycone. Zanthosides B and C (2, 3) were also prenylated phenylpropanoids, and the methyl ester in 2 may be formed during the extraction and isolation procedures.

Experimental

Experimental section is provided as a supplementary material through an internet system. https://www.jstage.jst.go.jp/article/cpb/00/00_c00-01012/_article.

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

**Supplementary Materials**  The online version of this article contains supplementary materials.

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