Musidunin and Musiduol, Insect Antifeedants from *Croton jatrophoides*

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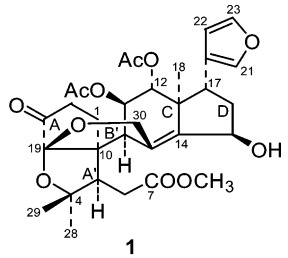
Two novel limonoids, musidunin (**1**) and musiduol (**2**), were isolated from a methanol extract of *Croton jatrophoides* by bioassay-guided fractionation. Their structures were established by extensive NMR experiments. Interestingly, A,B-seco limonoid **1** contains a unique acetal annulation of A, A′, and B′ rings. Both limonoids exhibited antifeedant activities against two pests, *Pectinophora gossypiella* and *Spodoptera frugiperda*.

Phytochemicals acting as deterrents to insect feeding are frequently found in pest-resistant plants, which can be lead compounds to develop environmentally friendly pest control agents.[1] In our continuing search for insect-affecting phytochemicals,[2−4] a bitter plant, *Croton jatrophoides* Pax. (Euphorbiaceae), which has been used as a folk medicine in East Africa, was identified to possess a highly pest-resistant property. Seven limonoids as insect antifeedant principals were isolated from a methanol extract of *C. jatrophoides*, and their structures, having a remarkable A−A′ ring closure, were determined by X-ray crystallographic and NMR analyses.[5−8] Limonoids are attractive biologically active substances because they possess anti-HIV activity,[9] antimalarial activity,[10] and cytotoxicity against cancer cell lines[11,12] in addition to insect antifeedant activity.[13,14] Systematic investigation of phytochemicals in *C. jatrophoides* was performed using antifeeding assays against two pests, the larvae of *Pectinophora gossypiella* and *Spodoptera frugiperda*. As a result, musidunin (**1**) and musiduol (**2**) were isolated as active constituents. Structural determinations and antifeedant potencies of **1** and **2** are described in this paper.



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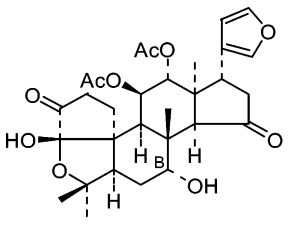

2

The methanol extract of the root bark of *C. jatrophoides* was partitioned between H_2O and *n*-hexane, chloroform, ethyl acetate, and *n*-butanol, in this order. The chloroform fraction showed strong insect antifeedant activity and was fractionated using silica gel column chromatography and preparative TLC. Compounds **1** and **2** were then isolated by preparative HPLC and were designated as musidunin and musiduol, respectively, based on the Swahili name of *C. jatrophoides*, "msinduzi".

Musidunin (**1**) was isolated as an amorphous solid. The molecular formula was unequivocally established as $C_{31}H_{38}O_{11}$ from the molecular ion peak at *m*/*z* 586 [M^+] by HREIMS analysis. The IR spectrum of **1** showed strong absorption near 1750 cm^{-1}, which indicated the presence of a carbonyl moiety. Aromatic methine signals (δ_H 7.34, 7.20, 6.23; δ_C 142.6, 140.0, 110.6) and a quaternary carbon signal at δ 122.6 in the ^1H and ^{13}C NMR spectra were assigned to a β-furan ring. Hence, this compound is classified as a limonoid.

The COLOC experiment revealed that the β-furan ring was attached to a methine (δ_H 3.04; δ_C 43.4) in the D ring (Figure S1, Supporting Information). The methine signal was connected to an oxymethine proton at δ 4.80 (H-15) via diastereotopic methylene protons at δ 2.27 and 1.92 (H-16) in the $^1H-^1H$ COSY spectrum. These protons were correlated with a quaternary carbon at δ 49.1 (C-13) that was bonded to the α-oriented methyl at H-18 (δ_H 1.01; δ_C 17.1) and to a quaternary olefin carbon at δ 145.0 (C-14) in the COLOC spectrum. The hydroxyl group at C-15 was deduced as β-oriented due to the NOE correlation between H-15 and H-18 (Figure S2, Supporting Information). C-13 possessed a COLOC correlation with an acetylated oxymethine (H-12; δ_H 5.36; δ_C 75.3) in the C ring. This oxymethine was coupled with an acetylated oxymethine (H-11; δ_H 5.63; δ_C 69.1) that was further connected to a methane (H-9; δ_H 3.16; δ_C 39.3) in the $^1H-^1H$ COSY spectrum. H-9 and H-11 are α-oriented, as indicated by NOE correlations with H-18. The cross-peak between H-9 and an olefin quaternary carbon (δ_C 127.9) in the COLOC spectrum indicated that the tetrasubstituted olefin (C-8 and C-14) was in the C ring. The two acetylated oxymethines in the C ring are often found in limonoids from *C. jatrophoides*, and the vicinal coupling constants are usually 0.8−5.1 Hz.[5−8] The large coupling constant (9.8 Hz) between H-11 and H-12 of **1** suggested that the vicinal acetoxy moieties possessed pseudoequatorial relationships on this cyclohexene ring.

The geminal dimethyls (δ_H 1.44, 1.30; δ_C 31.9, 26.1) identified by the $^{13}C-^1H$ COSY experiment were placed at C-28 and C-29 in the A′ ring as in dumnin.[7] In the COLOC experiment, H-28 was correlated to a methine (δ_H 2.81; δ_C 54.7) that was coupled with methylene protons at δ 2.59 and 2.31. This proton sequence was assigned to H-5 and H-6. However, from inspection of the COLOC correlations, C-6 was adjacent to a carboxymethyl moiety (δ_H 3.70; δ_C 52.1, 172.6) at C-7, indicating that the B ring in **1** was absent.

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Diastereotopic methylene protons at δ 2.97 and 2.12 were connected to methylene protons at δ 2.57 in the $^1H-^1H$ COSY spectrum. The signals of H-5, H-9, and its diastereotopic methylene (H-1) possessed cross-peaks to a quaternary carbon (C-10; δ_C 57.8). Also, COLOC correlations between the vicinal methylene signals (H-1 and H-2) and a carbonyl carbon (δ_C 210.7) and an oxygenated quaternary carbon (δ_C 108.8) confirmed the presence of the A ring. Thus, compound **1** contained the A and A′ rings as in other dumsin-type limonoids,[5,7] and the B ring was absent. The remaining NMR signals were due to an oxymethylene (δ_H 4.81, 4.31; δ_C 64.3). These methylene protons were correlated to olefin carbons (C-8 and C-14), the oxygenated quaternary carbon (C-19), and the methylene carbon (C-9). Thus, the oxymethylene was included in the B′ ring. Annulation of the A′, A, and B′ rings of **1** was supported by several key NOE correlations.

Musiduol (**2**) was isolated as an amorphous solid, and the molecular formula was established as $C_{30}H_{38}O_{10}$ by HRFABMS. An absorption at 1757 cm^{-1} in the IR spectrum indicated the presence of a carbonyl moiety. The ^1H and ^{13}C NMR spectra of **2** were similar to those of dumnin,[7] except for absence of signals originating from a phenylacetoxy moiety in the C ring and the trisubstituted epoxide in the D ring. Instead, carbonyl carbon (δ_C 219.9), isolated methine (δ_H 2.85; δ_C 57.9), and oxymethine (δ_H 3.98; δ_C 71.3) signals were identified by ^{13}C NMR, DEPT, and $^{13}C-^1H$ COSY experiments.

The oxymethine was assigned to H-7, as it was correlated to a methine (H-5; δ_H 2.61; δ_C 48.9) through a methylene (H-6; δ_H 2.22, 1.70; δ_C 28.2) in the $^1H-^1H$ COSY spectrum. The NOE correlation between H-7 and H-30 and the small coupling constant of H-7 (3.3 Hz) indicated that the OH group was α-oriented (Figure S3, Supporting Information). The isolated methine possessed COLOC cross-peaks with C-8, H-30, and H-18, indicating that the methine was in the D ring (C-14). The remaining carbonyl carbon was assigned to C-15. The α-orientation of H-14 was deduced from the NOE between H-14 and H-9 and H-18.

Ring D, having a carbonyl moiety, has been found in other limonoids.[16,17] As in **2**, and in other dumsin- and zumsin-type limonoids from *C. jatrophoides*, the D ring is resistant to oxidation. Moreover, C-30 and the B ring of **1** appear to be preferentially oxidized before A ring oxidation. Although many B ring oxidized limonoids have been reported,[18−20] compound **1** is classified as one of a very few examples with respect to this type of acetal annulation of A, A′, and B′ rings. One possibility of biogenesis of **1** from dumsenin[7] is illustrated in Figure S4 (Supporting Information).

Both limonoids **1** and **2** exhibited insect antifeedant activities (PC_{50} = 3 μg/mL, PC_{95} = 10 μg/mL; PC_{50} = 4 μg/mL, PC_{95} = 20 μg/mL, respectively) against the second-instar larvae of *P. gossypiella* in a leaf disk assay.[21] Compound **2** also possessed insect antifeedant activity against *S. frugiperda* with PC_{50} = 2.0 μg/mL and PC_{95} = 36 μg/mL. Insect antifeedant activity of **1** was also observed, although **1** could not be isolated in sufficient quantity for a detailed assay. The two pests *P. gossypiella* and *S. frugiperda* damage cottonseed, corn, and other commercial crops produced in countries such as the United States, India, China, and Australia.[22,23] Therefore, unique limonoids such as **1** and **2** may be useful as pest control agents due to their potent insect antifeedant activities.

Experimental Section

General Experimental Procedures. Specific rotations were recorded in MeOH on a JASCO DIP-370 digital polarimeter (Tokyo, Japan). IR spectra were recorded in $CHCl_3$ on a Horiba FT-720 spectrometer (Kyoto, Japan). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as internal reference on JEOL JNM-GX-400 and EX-400 spectrometers (Akishima, Japan). HREIMS and HRFABMS were measured in the positive-ion mode on a JEOL JMS-700TKM spectrometer. Preparative TLC plates were purchased from Analtech, Inc. (Newark, DE). All solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Preparative HPLC was performed with an EYELA LPG-1000 instrument and an EYELA UV7000 detector (Tokyo Rikakikai Co. Ltd., Tokyo, Japan), on a 10 mm × 250 mm i.d., 10 μm, Alltech Econosil C_{18} column (Deerfield, IL). Initially, 40% MeCN/H_2O was used as the HPLC solvent. The gradient elution was started at 5 min and proceeded from 40% to 80% MeCN/H_2O in 30 min. For the isolation of **1**, isocratic elution with 45% MeCN/H_2O was then performed. The flow rate and detected wavelength were adjusted to 5 mL/min and 210 nm, respectively.

Plant Material. Root bark of the East African medicinal plant locally known as "msinduzi" was collected near Mombasa, Kenya, and the plant was identified as *C. jatrophoides* (Euphorbiaceae).[24] A plant specimen (AC 76-134) was deposited in the Department of Botany herbarium at the University of Nairobi.

Extraction and Isolation. The root bark was removed at the collection site. The air-dried root bark (500 g) was cut into small pieces and extracted with MeOH (500 mL × 3) at ambient temperature for 2 weeks. The solvent was evaporated in vacuo, and the resulting residue (40 g) was partitioned between H_2O (800 mL) and *n*-hexane (200 mL × 3), $CHCl_3$ (200 mL × 3), and EtOAc (200 mL × 3), respectively. A leaf disk assay against second-instar larvae of *P. gossypiella* identified the $CHCl_3$ fraction (4.5 g) as containing the antifeedant activity. This fraction was further divided into six fractions (I: 0.2 g, II: 0.7 g, III: 0.8 g, IV: 0.4 g, V: 1.1 g, and VI: 0.5 g) using chromatography on silica gel (70−230 mesh, 250 g) eluted with 1−20% MeOH/$CHCl_3$. Subsequent bioassays showed moderate activity in the 20% MeOH/$CHCl_3$ eluted fraction (V). This fraction was subjected to preparative TLC with 20% MeOH/$CHCl_3$ and then further purification by preparative HPLC to give 1.8 mg of **1** (t_R = 15.0 min by the isocratic mode) and 2.5 mg of **2** (t_R = 17.0 min).

Musidunin (1): colorless solid; $[\alpha]^{24}_D$ −40.6 (*c* 0.09, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1747, 1371, 1245 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34 (1H, m, H-23), 7.20 (1H, bs, H-21), 6.23 (1H, m, H-22), 5.63 (1H, dd, *J* = 9.8, 7.3 Hz, H-11), 5.36 (1H, d, *J* = 9.8 Hz, H-12), 4.81 (1H, d, *J* = 15.0 Hz, H-30a), 4.80 (1H, d, *J* = 7.8 Hz, H-15), 4.31 (1H, d, *J* = 15.0 Hz, H-30b), 3.70 (3H, s, COOC*H*$_3$), 3.16 (1H, d, *J* = 7.3 Hz, H-9), 3.04 (1H, dd, *J* = 12.4, 6.8 Hz, H-17), 2.97 (1H, ddd, *J* = 13.7, 9.3, 4.4 Hz, H-1a), 2.81 (1H, dd, *J* = 11.7, 3.4 Hz, H-5), 2.59 (1H, m, H-6a), 2.57 (2H, m, H-2), 2.31 (1H, dd, *J* = 16.1, 3.4 Hz, H-6b), 2.27 (1H, ddd, *J* = 14.6, 12.4, 7.8 Hz, H-16a), 2.12 (1H, dt, *J* = 13.7, 9.8 Hz, H-1b), 2.05 (3H, s, OCOC*H*$_3$), 1.92 (1H, dd, *J* = 14.6, 6.8 Hz, H-16b), 1.75 (3H, s, OCOC*H*$_3$), 1.61 (1H, bs, O*H*), 1.44 (3H, s, H-28), 1.30 (3H, s, H-29), 1.01 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.7 (C, C-3), 172.6 (C, C-7), 171.43 (C, O*C*OCH$_3$), 171.39 (C, O*C*OCH$_3$), 145.0 (C, C-14), 142.6 (CH, C-23), 140.0 (CH, C-21), 127.9 (C, C-8), 122.6 (C, C-20), 110.6 (CH, C-22), 108.8 (C, C-19), 89.9 (C, C-4), 75.3 (CH, C-12), 69.1 (CH, C-11), 68.5 (CH, C-15), 64.3 (CH_2, C-30), 57.8 (C, C-10), 54.7 (CH, C-5), 52.1 (CH_3, COO*C*H$_3$), 49.1 (C, C-13), 43.4 (CH, C-17), 41.0 (CH_2, C-16), 39.3 (CH, C-9), 34.4 (CH_2, C-6), 34.3 (CH_2, C-2), 31.9 (CH_3, C-28), 30.1 (CH_2, C-1), 26.1 (CH_3, C-29), 21.0 (CH_3, OCO*C*H$_3$), 20.5 (CH_3, OCO*C*H$_3$), 17.1 (CH_3, C-18); HREIMS *m*/*z* 586.2394 $[M]^+$ (calcd for $C_{31}H_{38}O_{11}$, 586.2414).

Musiduol (2): colorless solid; $[\alpha]^{25}_D$ −24.2 (*c* 0.12, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1757, 1734, 1371, 1230 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (1H, m, H-23), 7.17 (1H, bs, H-21), 6.20 (1H, m, H-22), 5.40 (1H, d, *J* = 2.8 Hz, H-12), 4.91 (1H, t, *J* = 2.8 Hz, H-11), 3.98 (1H, d, *J* = 3.3 Hz, H-7), 3.67 (1H, t, *J* = 9.2 Hz, H-17), 3.56 (1H, bs, O*H*), 2.85 (1H, bs, H-14), 2.66 (1H, m, H-2a), 2.64 (1H, dd, *J* = 11.2, 9.2 Hz, H-16a), 2.61 (1H, dd, *J* = 9.6, 5.4 Hz, H-5), 2.50 (1H, d, *J* = 2.8 Hz, H-9), 2.48 (1H, dd, *J* = 11.2, 9.2 Hz, H-16b), 2.35 (1H, m, H-2b), 2.30 (1H, m, H-1a), 2.22 (1H, dd, *J* = 13.0, 9.6 Hz, H-6a), 2.06 (3H, s, OCOC*H*$_3$), 2.05 (3H, s, OCOC*H*$_3$), 1.75 (1H, ddd, *J* = 14.0, 10.4, 2.0 Hz, H-1b), 1.70 (1H, ddd, *J* = 13.0, 5.4, 3.3 Hz, H-6b), 1.61 (1H, bs, O*H*), 1.45 (3H, s, H-29), 1.40 (3H, s, H-30), 1.36 (3H, s, H-28), 0.78 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 219.9 (C, C-15), 210.0 (C, C-3), 171.0 (C, O*C*OCH$_3$), 169.5 (C, O*C*OCH$_3$), 143.6 (CH, C-23), 139.9 (CH, C-21), 122.9 (C, C-20), 110.4 (CH, C-22), 104.9 (C, C-19), 85.3 (C, C-4), 71.5 (CH, C-11), 71.3 (CH, C-7), 71.2 (CH, C-12), 57.9 (CH, C-14), 55.7 (C, C-10), 48.9 (CH, C-5), 44.5 (C, C-13), 43.5 (CH, C-9), 43.1 (CH_2, C-16), 39.5 (C, C-8), 37.9 (CH, C-17), 35.2 (CH_2, C-1), 32.0 (CH_3, C-29), 31.5 (CH_2, C-2), 28.2 (CH_2, C-6), 28.1 (CH_3, C-28), 21.9 (CH_3, OCO*C*H$_3$), 21.5 (CH_3, C-18), 20.9 (CH_3, OCO*C*H$_3$), 19.4 (CH_3, C-30); HRFABMS *m*/*z* 559.2545 $[M + H]^+$ (calcd for $C_{30}H_{39}O_{10}$, 559.2543).

Insect Antifeedant Assay. Second-instar larvae of *P. gossypiella* and *S. frugiperda* were used as test organisms, and a leaf disk assay was performed by the method reported previously.[21] Briefly, leaf disks (1 cm^2) were punched out from a glandless cotton cultivar, randomized, and arranged (12 disks/dish) concentrically on moistened filter paper within polyethylene foam grids inside glass Petri dishes (100 mm × 15 mm). Alternate disks were treated on their upper surface with either 25 μL of acetone or 0−100 μg of the sample dissolved in 25 μL of acetone applied with a microliter syringe. Three larvae were then placed in the dishes at 22 °C in a dark incubator. After 48 h, the larvae were removed and disks were examined visually. Percent area of the leaf disk consumed versus control was recorded. PC_{50} and PC_{95} values are the concentrations at which the test compounds afforded ca. 50% and 95% protection of the host plant substrate, respectively. The assays were performed in triplicate, on separate occasions, and their range of error was within 0.5 μg/mL.

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Supporting Information Available: Selected NOE and COLOC correlations for **1** and **2** and possible biogenetic pathway of **1** from dumsenin. This material is available free of charge via the Internet at http://pubs.acs.org.

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