Insect Antifeedants from *Croton jatrophoides*: Structures of Zumketol, Zumsenin, and Zumsenol†

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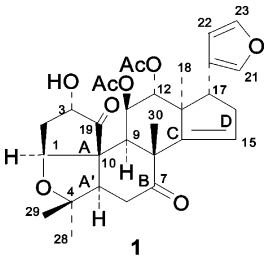
Three new A-seco limonoids, zumketol (**1**), zumsenin (**2**), and zumsenol (**3**), were isolated from a methanol extract of *Croton jatrophoides* by bioassay-guided fractionation, and their structures were determined by NMR analysis. The α-ketol **1** may be biosynthesized via the intramolecular acyloin condensation of a dicarboxylate intermediate.

In our continuing search for biologically active substances from tropical plants, the structures of the novel limonoids dumsin, zumsin, dumnin, and dumsenin were previously reported.[1−3] These limonoids are unusual with respect to their A−A′ ring structure and have potent antifeedant activity against two pests, the larvae of *Pectinophora gossypiella* and *Spodoptera frugiperda*. In addition to their insect antifeedant activity, limonoids have attracted much attention since they exhibit several biological effects such as antimalarial activity[4,5] and cytotoxicity against cancer cell lines.[6−8] In this report, we describe the isolation, structure determination, and insect antifeedant activity of three new limonoids, zumketol (**1**), zumsenin (**2**), and zumsenol (**3**), from *Croton jatrophoides* Pax. (Euphorbiaceae), which has been used as a folk medicine in East Africa.

The root bark of *C. jatrophoides* was extracted with MeOH, and the extract was subsequently partitioned between water and several organic solvents. The chloroform fraction, having strong insect antifeedant activity against the larvae of *P. gossypiella*, was purified by silica gel column chromatography and preparative TLC. Finally, preparative HPLC led to the isolation of three compounds, **1**−**3**, which were designated as zumketol, zumsenin, and zumsenol, respectively, based on the Swahili name of *C. jatrophoides*, "msinduzi".

Compound **1** was isolated as an amorphous solid, and the molecular formula was established as $C_{30}H_{36}O_9$ by HREIMS analysis. The IR spectra displayed absorptions due to carbonyl groups at 1745 cm^{-1}. This compound was deduced as being a tetranortriterpenoid by taking into account the total carbon number (C_{30}) and two acetyl groups (δ_H 1.81, 1.98; δ_C 21.1, 22.4, 171.2, 171.8) by ^1H and ^{13}C NMR and HRMS experiments. Signals at 7.35 and 6.23 ppm (H-23 and H-22) showed cross-peaks to each other in the $^1H-^1H$ COSY spectrum, whereas a signal at 7.15 ppm (H-21) was isolated. These signals were assigned to a β-furan structure (δ_C 111.4, 124.1, 140.4, 142.5), indicating that compound **1** can be classified as a limonoid.

An olefinic methine proton at 6.46 ppm (H-15) was connected through the methylene protons at 2.50 ppm (H-16) with a methine signal at 3.05 ppm. To this methine (H-17), the β-furan ring was attached. Accordingly, those three resonances were sited in the D ring, having a trisubstituted olefin (δ_C 129.6, 149.6), as previously shown in the structure of several limonoids.[9,10] Two acetylated oxymethine protons at 5.26 and 5.16 ppm (H-12 and H-11) were correlated to each other in the $^1H-^1H$ COSY spectrum. The signal at H-11 was coupled to a methine proton at 2.97 ppm (H-9), indicating that two acyloxy groups were located in the C ring as in the structures of dumsin and related compounds.[1−3] In the $^{13}C-^1H$ COSY spectrum, a carbon signal at 36.1 ppm (C-6) was correlated to geminal protons at 2.36 and 3.42 ppm (H-6). Further coupling of these methylene protons and a methine proton at 2.33 ppm (H-5) permitted the construction of the B ring structure proposed. An oxymethine proton at 4.39 ppm was assigned to H-1 since the chemical shifts of this proton and corresponding carbon (δ_C 83.5) were similar to those of zumsin. An oxymethine proton at 4.47 ppm (H-3) was correlated to H-1 via the methylene protons at 1.85 and 2.57 ppm.



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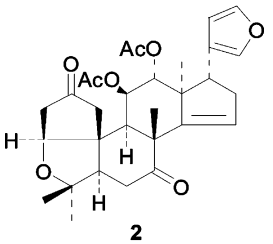

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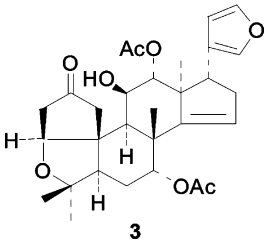

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† In honor of Professor Tadao Kamikawa's 70th birthday.

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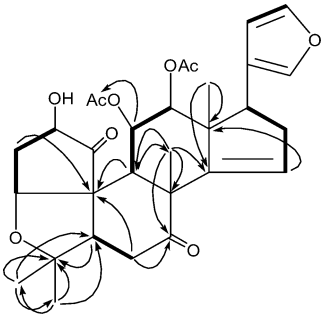

Figure 1. Selected ^{1}H−^{1}H COSY (—) and COLOC (→) correlations for **1**.

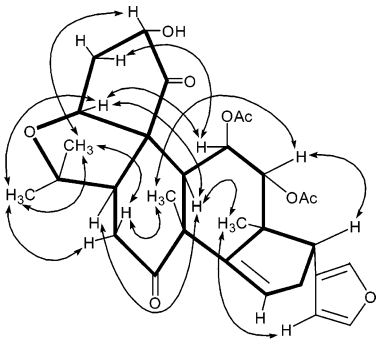

Figure 2. Significant NOE correlations for **1**.

Hence, their four signals were ascribed to the A ring structure. All of the proton and carbon sequences, by observing ^{1}H−^{1}H and ^{13}C−^{1}H COSY experiments, were connected by COLOC experiments, as shown in Figure 1.

The relative stereochemistry of **1** was determined by a NOESY experiment and analysis of proton coupling constants. Significant NOE correlations are shown in Figure 2. A cross-peak between the methine proton at C-17 and the oxymethine proton at C-12 was consistent with an α-oriented acetyl group at C-12, on the basis of the assumption of an α-oriented furan ring in the limonoid skeleton. Likewise, the proton at 6.23 ppm (H-22) on the furan ring was correlated to α-oriented methyl protons at C-18 (δ_H 1.09; δ_C 17.0). The C ring conformation could be defined as a boat form due to the observations of the NOEs between the flag-pole protons (H-18 and H-9) and between the β-oriented oxymethine proton at C-12 and the methyl protons at C-30, and a small vicinal coupling constant (J = 4.4 Hz) between H-12 and H-11. The 1,3-diaxial protons, H-9 and H-5, possessed a NOE correlation so that both are α-oriented. The deshielded methylene proton at 3.42 ppm was assigned as β because of the large coupling constant with H-5. In addition, the presence of a NOE between H-6β and H-30 ascertained that the B ring conformation was in the chair form. The oxymethine proton at C-1 had three cross-peaks with the α-oriented H-11, H-9, and H-28 in the NOESY spectrum. Also, the deshielded chemical shifts at H-6β and H-30 indicated the carbonyl moiety was located close to those protons. Hence, the A−A′ ring junction of **1** was identical to that of zumsin. The stereochemistry of the hydroxyl group at C-3 was assigned as α, which was deduced from a NOE correlation between H-3 and H-29.

Compound **2** was isolated as an amorphous solid, and the molecular formula was determined as $C_{30}H_{36}O_8$ by a HREIMS experiment. This structure possesses an isolated methylene (δ_H 2.64, 3.13; δ_C 41.5) and two oxymethines (δ_H 5.23; δ_C 76.1, 83.1), which are also found in the structure of zumsin. The two oxymethines were not clearly resolved. However, an observed NOE between these signals and H-17 was consistent with an α-acetoxy group at C-12 (Figure S1, Supporting Information). In addition, a boat form of the C ring was deduced from a NOE between H-9 and H-18. The B ring was observed to be in a chair form since the α-oriented H-5 possessed small and large coupling constants (J = 2.8 and 14.0 Hz). Two cross-peaks between H-1 and H-29 and between H-8 and H-19α in the NOESY spectrum supported the stereochemistry of the A ring being similar to that of **1**. Accordingly, the structure of **2** was ascribed as the precursor of zumsin before epoxidation on the D ring.

Compound **3** was isolated as an amorphous solid, and the molecular formula was established as $C_{30}H_{38}O_8$ by a HRFABMS experiment. The ^{1}H and ^{13}C NMR spectra were similar to those of **1**, apart from the presence of a carbonyl carbon (δ_C 217.3) and an isolated methylene (δ_H 2.31, 3.29; δ_C 42.5). The ^{1}H and ^{13}C NMR assignments for **3** were performed with the aid of ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, COLOC, and NOESY experiments (Figures S2 and S3, Supporting Information). The oxymethine proton at 4.69 ppm (H-12) had correlations with a methine proton at 2.48 ppm (H-9) via a shielded oxymethine proton at 3.92 ppm (H-11) in the ^{1}H−^{1}H COSY spectrum, indicating that these protons and the corresponding carbons were assigned to the C ring. This conformation was defined in the boat form since two NOEs were exhibited between H-9 and H-18 and between H-12 and H-30. The B ring structure was constructed by considering sequential cross-peaks among H-5 (δ_H 2.27), H-6 (δ_H 1.77 and 1.90), and H-7 (δ_H 5.22) in the ^{1}H−^{1}H COSY spectrum. The small coupling constants (J = 2.2 and 2.9 Hz) of the oxymethine proton at H-7 were clarified due to the attached oxygen being in an α-orientation. Likewise, H-5 was α due to the presence of small and large coupling constants (J = 2.9 and 13.9 Hz). The NOEs observed between H-6β and H-30 and between H-7 and H-30 were consistent with a chair form of the B ring. The H-1 signal had two correlations with H-9 and H-10 in the NOESY spectrum. These significant cross-peaks indicated that the A−A′ ring junction was similar to that of **1**. Further investigation was still needed to determine the location of the hydroxylated oxymethine in the structure of **3**. The shielded H-11 oxymethine (δ_H 3.92) and observed NOE between the broadened hydroxyl signal (δ_H 4.00) and an acylated oxymethine signal (δ_H 4.69) suggested that the hydroxyl group is located at the C-11 position.[11] The ^{1}H and ^{13}C NMR assignments for **1**−**3** are given in Table 1.

Limonoids **1**−**3**, as well as previously reported limonoids of the same type,[1−3] are rare examples with respect to the oxidation of the A ring prior to that of the D ring. In addition, the A−A′ ring formation possibly occurred before the olefinic oxidation on the D ring because this plant extract contained **2** and zumsin. The branched biosynthetic pathway after the A ring expansion by Baeyer−Villiger oxidation was discussed in a previous report.[2] Finding the zumsin-type limonoids **1**−**3** in *C. jatrophoides* supported this conjecture, and moreover, the presence of the α-ketol **1** adds additional information on their unique A−A′ ring formation. It can be proposed that zumsin-type limonoids are biosynthesized via intramolecular acyloin condensation of a dicarboxylate intermediate (Figure S4, Supporting Information).[12] This process would be expected to be involved in the biogenetic pathway of dumsin-type limonoids.

Insect antifeedant activity of **1** was evaluated against the second-instar larvae of *P. gossypiella* and *S. frugiperda*, although **2** and **3** could not be tested due to the limited amounts available. Compound **1** showed potent activities with PC_{50} = 0.5 μg/mL and PC_{90} = 2 μg/mL against *P.*

Table 1. ^{1}H and ^{13}C NMR Data for **1**−**3** in $CDCl_3$

	1		**2**		**3**	
position	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)
1	4.39 (d, 2.9)	83.5 (d)	4.37 (d, 3.6)	84.4 (d)	4.32 (d, 3.0)	84.7 (d)
2	1.85 (ddd, 2.9, 10.3, 13.2)	33.0 (t)	2.39 (dd, 3.6, 18.4)	45.2 (t)	2.37 (d, 17.6)	45.7 (t)
	2.57 (dd, 8.8, 13.2)		2.44 (d, 18.4)		2.65 (dd, 3.0, 17.6)	
3	4.47 (dd, 8.8, 10.3)	76.7 (d)		216.5 (s)		217.3 (s)
4		80.9 (s)		80.8 (s)		80.7 (s)
5	2.33 (dd, 2.8, 13.2)	60.7 (d)	2.19 (dd, 2.8, 14.0)	59.9 (d)	2.27 (dd, 2.9, 13.9)	54.4 (d)
6	2.36 (dd, 2.8, 16.1)	36.1 (t)	2.44 (dd, 2.8, 15.6)	38.1 (t)	1.77 (td, 2.9, 13.9)	26.0 (t)
	3.42 (dd, 13.2, 16.1)		2.79 (dd, 14.0, 15.6)		1.90 (dt, 2.2, 13.9)	
7		207.9 (s)		206.8 (s)	5.22 (dd, 2.2, 2.9)	75.8 (d)
8		50.4 (s)		51.3 (s)		43.0 (s)
9	2.97 (d, 5.1)	49.9 (d)	2.80 (d, 3.6)	47.6 (d)	2.48 (d, 5.1)	45.0 (d)
10		57.2 (s)		53.5 (s)		53.6 (s)
11	5.16 (dd, 4.4, 5.1)	74.3 (d)	5.23 (m)	76.1 (d)	3.92 (dd, 4.4, 5.1)	75.4 (d)
12	5.26 (d, 4.4)	82.0 (d)	5.23 (m)	83.1 (d)	4.69 (d, 4.4)	91.0 (d)
13		51.3 (s)		51.3 (s)		50.3 (s)
14		149.6 (s)		147.9 (s)		154.5 (s)
15	6.46 (dd, 2.2, 3.0)	129.6 (d)	6.35 (dd, 1.8, 2.4)	131.4 (s)	5.64 (m)	124.0 (s)
16	2.50 (m)	37.5 (t)	2.52 (m)	37.8 (t)	2.39 (m)	36.6 (t)
	2.50 (m)		2.52 (m)		2.51 (m)	
17	3.05 (t, 9.5)	50.7 (d)	3.06 (t, 9.6)	50.8 (d)	3.00 (dd, 8.1, 11.0)	51.5 (d)
18	1.09 (s)	17.0 (q)	1.09 (s)	17.2 (q)	1.03 (s)	16.0 (q)
19		219.8 (s)	2.64 (d, 19.2)	41.5 (t)	2.31 (d, 19.1)	42.5 (q)
			3.13 (d, 19.2)		3.29 (d, 19.1)	
20		124.1 (s)		124.4 (s)		124.3 (s)
21	7.15 (bs)	140.4 (d)	7.16 (bs)	140.7 (d)	7.25 (bs)	140.4 (d)
22	6.23 (m)	111.4 (d)	6.23 (m)	111.7 (d)	6.24 (m)	111.8 (d)
23	7.35 (m)	142.5 (d)	7.34 (t, 1.6)	142.7 (d)	7.37 (m)	142.2 (d)
28	1.26 (s)	29.8 (q)	1.29 (s)	31.5 (q)	1.23 (s)	31.3 (q)
29	0.97 (s)	23.3 (q)	1.18 (s)	24.1 (q)	1.12 (s)	23.4 (q)
30	1.74 (s)	30.2 (q)	1.58 (s)	30.6 (q)	1.39 (s)	29.0 (q)
OH-3	1.57 (bs)					
OH-11					4.00 (bs)	
OAc-7					2.22 (s)	21.2 (q)
						169.6 (s)
OAc-11	1.81 (s)	22.4 (q)	1.83 (s)	21.4 (q)		
		171.2 (s)		171.3 (s)		
OAc-12	1.98 (s)	21.1 (q)	1.99 (s)	21.6 (q)	2.23 (s)	21.1 (q)
		171.8 (s)		171.2 (s)		173.7 (s)

gossypiella, and PC_{50} = 3 μg/mL and PC_{90} = 16 μg/mL against *S. frugiperda*, which were similar to that of previously reported limonoids of the same type.[2] To investigate more details of the structure−activity relationships and the biogenetic pathway of the limonoids, additional research is suggested using *C. jatrophoides* as an interesting plant source.

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). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as internal reference on a JEOL JNM-GX-400 spectrometer (Akishima, Japan). HREIMS and HRFABMS were measured in the positive-ion mode on a JEOL JMS-700TKM spectrometer. Preparative HPLC was performed in the gradient mode 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 the solvent composition was changed to 80% MeCN/H_2O in 30 min. The flow rate and detected wavelength were adjusted at 5 mL/min and 210 nm, respectively. Preparative TLC plates were purchased from Analtech, Inc. (Newark, DE). All solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Plant Material. The 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).[13] The 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 peeled off 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 then the resulting residue (40 g) was partitioned between water (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 strong activity in the 10% MeOH/$CHCl_3$ eluted fraction (IV). This fraction was subjected to preparative TLC with 15% MeOH/$CHCl_3$, and then further purification by preparative HPLC give 20 mg of **1** (t_R = 22.0 min), 12 mg of **2** (t_R = 25.0 min), and 7 mg of **3** (t_R = 23.0 min).

Zumketol (1): colorless solid; $[\alpha]^{25}_D$ −38.3° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1745, 1716, 1371, 1230 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 540.2321 $[M]^+$ (calcd for $C_{30}H_{36}O_9$, 540.2359).

Zumsenin (2): colorless solid; $[\alpha]^{25}_D$ −66.6° (*c* 1.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1749, 1718, 1373, 1232 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 524.2427 $[M]^+$ (calcd for $C_{30}H_{36}O_8$, 524.2410).

Zumsenol (3): colorless solid; $[\alpha]^{25}_D$ −68.8° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1739, 1720, 1373, 1251 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 527.2673 $[M + H]^+$ (calcd for $C_{30}H_{39}O_8$, 527.2645).

Insect Antifeedant Assay. Second-instar larvae of *P. gossypiella* and *S. frugiperda* were used as the test organisms, and a leaf disk assay was performed by the method as previously reported.[14] 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 as percent area of the leaf disk consumed versus control. 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.

Acknowledgment. We are indebted to the late Dr. J. A. Klocke for performing the leaf disk assay and to Mr. A. Chapya for collection and identification of the plant material.

Supporting Information Available: Significant COLOC and NOE correlations for **2** and **3**, and proposed biogenetic pathway in zumsin-type limonoids. This material is available free of charge via the Internet at http://pubs.acs.org.

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