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J. Nat. Prod., **1991**, 54 (1), 241-246• DOI: 10.1021/np50073a025 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 1, pp. 241-246, Jan-Feb 1991

LIMONOID ANTIFEEDANTS FROM SEED OF *SANDORICUM KOETJAPE*[1]

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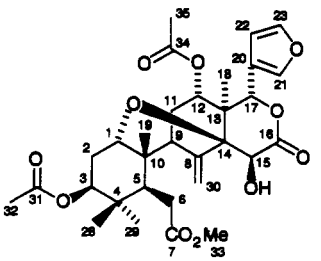
ABSTRACT.—An extract of the seed of *Sandoricum koetjape* has yielded two new limonoids, sandoricin [**1**] and 6-hydroxysandoricin [**2**]. Both compounds are effective antifeedants when incorporated into artificial diets and fed to fall armyworm (*Spodoptera frugiperda*) or European corn borer (*Ostrina nubilalis*) larvae. Reduced growth rates and increased times to pupation were evident at lower dose levels while significant mortality was noted at higher dose levels. Structures of both compounds were determined by ^{1}H nmr, ^{13}C nmr, and ms and confirmed by X-ray crystallography.

Continuing our studies of lesser-known species of the family Meliaceae in search of new natural products with activity in insects (1–3), we now report a detailed examination of seed extracts of *Sandoricum koetjape* Merr. [syn. *Sandoricum indicum* Cav. Santol]. *S. koetjape* is a medium-sized tree native to southeast Asia, including Malaya and the Philippine Islands, and bears edible fruit that is consumed by natives (4). Previous chemical studies of this species include reports of fatty acid composition of the seed oil (5) and triterpenoid constituents of the fruit hulls (6). Antifeedant-activity-directed fractionation of the seed extract, utilizing larvae of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), and European corn borer, *Ostrina nubilalis* (Hübner) (Leptidoptera: Pyralidae), has resulted in the isolation of two new limonoids, sandoricin [**1**] and 6-hydroxysandoricin [**2**], as the primary active constituents.

Antifeedant-activity-directed isolation of **1** and **2** was accomplished by extracting the ground seed material with solvent (hexane and EtOH) and subjecting the extracts to repeated cc on Si gel followed by preparative hplc. Both compounds were obtained as colorless crystalline solids. The cims of **1** and **2** exhibited apparent protonated molecular ions at *m/z* 589 and *m/z* 605, respectively. Hreims of **1** gave $[M]^+$ *m/z* 588.2570 ($C_{31}H_{40}O_{11}$), and hreims of **2** gave $[M]^+$ *m/z* 604.2525 ($C_{31}H_{40}O_{12}$). Compounds **1** and **2** were closely related in structure as evidenced by comparison of their ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2). Obvious similarities include four quaternary methyl



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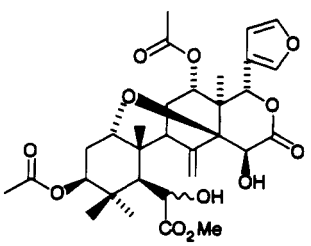

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[2]USDA, ARS, retired.

TABLE 1. ^{1}H-nmr Assignments for Sandoricin [**1**] and 6-Hydroxysandoricin [**2**].[a]

Proton	Compound	
	1	**2**
H-1	3.71 dd (2.4, 4.2)	3.75 dd (2.8, 3.5)
H-2a	1.90 ddd (2.4, 4.0)	1.87 m
H-2b	1.78 ddd (4.2, 12.6)	1.87 m
H-3	4.95 dd (4.0, 12.6)	4.89 dd (5.4, 10.9)
H-5	2.56 bd (10.8)	2.53 bs
H-6a	2.44 dd (10.8, 15.0)	4.13 d (2.8)
H-6b	1.96 bd (15.0)	—
6-OH	—	3.18 d (2.8)
H-9	2.17 dd (1.5, 5.1)	2.34 bd (3.9)
H-11a	2.32 ddd (1.5, 5.6, 14.0)	2.22 m (5.3, 14.3)
H-11b	1.38 m (5.1, 11.9, 14.0)	1.40 m
H-12	5.40 dd (5.6, 11.9)	5.33 (5.6, 11.9)
H-15	4.61 bd (2.2)	4.63 d (1.8)
15-OH	3.34 d	3.30 d (1.8)
H-17	5.78 s	5.79 s
Me-18	1.23 s	1.23 s
Me-19	0.80 s	0.88 s
H-21	7.32 m (1.7)	7.33 m
H-22	6.43 m (1.7)	6.43 m
H-23	7.41 m (1.4, 1.7)	7.42 bs
Me-28	0.86 s	1.19 s
Me-29	0.93 s	1.19 s
H-30a	5.31 s	5.37 s
H-30b	5.18 s	5.22 s
Me-32	1.99 s	2.00 s
Me-33	3.57 s	3.70 s
Me-35	1.47 s	1.47 s

[a]Nmr spectra were obtained with a Bruker WM-300 instrument in $CDCl_3$ solutions with TMS as an internal standard. Chemical shifts (δ) are expressed in ppm from TMS, and coupling constants J, in parentheses, are expressed in Hz.

singlets (H-18, -19, -28, and -29), two acetate methyl singlets (H-32 and -35), a methyl ester singlet (H-33), three olefinic protons characteristic of a furan moiety (H-21, -22, -23) and an exchangeable proton (15-OH). The additional oxygen of compound **2**, apparent from the ms, was present as a hydroxyl group evidenced by a second exchangeable proton at δ 3.18 (6-OH). Although ^{1}H- and ^{13}C-nmr spectra of **1** and **2** were consistent with the structures shown, additional information was required for complete elucidation of structures. The structures of **1** and **2** were determined unequivocally by single crystal X-ray crystallography. Compounds **1** and **2** are closely related structurally to several previously known limonoids, including methyl angolensate and ekbergin (7,8).

Compounds **1** and **2** both show significant antifeedant activity against fall armyworm larvae in a diet-incorporated bioassay at 25 ppm and similar activity against European corn borer larvae at 200 ppm (Table 3). Toxicity results for both insects indicate that continued feeding on diets containing either **1** or **2** at the 200-ppm level would result in near 100% mortality prior to pupation.

TABLE 2. ^{13}C-nmr Assignments for Sandoricin [**1**] and 6-Hydroxysandoricin [**2**].[a]

Carbon	Compound		Carbon	Compound	
	1	**2**		**1**	**2**
C-1	78.3 d	78.8 d	C-17	79.0 d	78.9d
C-2	28.7 t	29.3 t	C-18	9.8 q	9.7 q
C-3	74.2 d	74.4 d	C-19	21.8 q	25.9 q[b]
C-4	38.5 s	39.1 s	C-20	120.7 s	120.7 s
C-5	42.3 s	47.6 d	C-21	142.2 d	142.3 d
C-6	33.3 t	71.1 d	C-22	110.2 d	110.2 d
C-7	173.8 s	177.7 s	C-23	142.8 d	142.8 d
C-8	140.8 s	141.0 s	C-28	26.7 q	23.9 q[b]
C-9	49.7 d	50.2 d	C-29	15.4 q	18.0 q
C-10	42.9 s	43.8 s	C-30	114.7 t	114.9 t
C-11	29.9 t	30.0 t	C-31	169.0 s	169.0 s
C-12	69.3 d	69.0 d	C-32	21.1 q	21.0 q
C-13	47.1 s	47.2 s	C-33	51.6 q	52.9 q
C-14	80.3 s	82.7 s	C-34	170.0 s	170.0 s
C-15	68.7 d	68.8 d	C-35	20.0 q	20.0 q
C-16	174.2 s	174.3 s			

[a]Nmr spectra were obtained with a Bruker WM-300 instrument in $CDCL_3$ solutions with TMS as an internal standard, and multiplicities were confirmed in DEPT experiments. Chemical shifts (δ) are expressed in ppm from TMS. Assignments were confirmed utilizing COSY and 2D heteronuclear correlation experiments.

[b]Assignmenmts may be interchanged.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns block and are uncorrected. Hplc was carried out on a Spectra-Physics SP8700XR attached to two detectors: a Waters R401 differential refractometer and an LCD/Milton Roy spectroMonitor D variable wavelength (UV 254) detector. Separations were performed using Rainin Dynamax silica columns: 21.4 mm i.d. × 5 cm, 3% MeOH/$CHCl_3$; or 10 mm i.d. × 25 cm, 1% MeOH/$CHCl_3$.

PLANT MATERIAL AND EXTRACTION.—Seeds of *S. koetjape* stored at the Northern Regional Research Center, Peoria, Illinois, under the identifier NU62560, were collected in Thailand and authenti-

TABLE 3. Antifeedant Bioassay Results for Compounds **1** and **2**.[a]

Compound	Concentration (ppm)						
	1600	800	400	200	100	50	25
Fall armyworm (*Spodoptera frugiperda*)							
1	0.01[b]	0.01[b]	0.00[b]	0.10[b]	0.09[b]	0.14b	0.43[b]
2	0.04[b]	0.05[b]	0.04[b]	0.03[b]	0.13[b]	0.34[b]	0.37[b]
European corn borer (*Ostrina nubilalis*)							
1	0.02[b]	0.07[b]	0.17[b]	0.34[b]	0.72	1.05	1.05
2	0.05[b]	0.02[b]	0.22[b]	0.30[b]	0.62	0.98	0.86

[a]Feeding ratio = number of larvae on treated discs/number of larvae on control discs. Number of larvae used to determine data points ranged from 58 to 97.

[b]Indicates significant difference at the 0.001 level by Chi-square analysis.

cated by USDA botanists, Beltsville, Maryland. An additional sample (8 kg) was collected in 1985 and purchased from the National Genebank of Thailand, Thailand Institute of Scientific and Technological Research, Bangkhen, Bangkok 10900.

Ground seed kernels (114 g) were extracted with hexane in a Soxhlet extractor for 8 h; the remaining seed meal was air-dried and then extracted for 8 additional hours with 95% EtOH. The hexane extract yielded 2.1 g of soluble material, and the EtOH extract yielded 13.7 g of soluble material.

FRACTIONATION OF EXTRACT.—Both the hexane and EtOH extracts were initially subjected to cc on a 72 × 6-cm glass column packed with Si gel. Eluting solvents were: $CHCl_3$; then 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50% MeOH in $CHCl_3$; then MeOH. Fractions with antifeedant activity were combined, and activity was further enriched by preparative hplc. Final preparative cleanup, 30 mg per injection, was done using 10 or 20% *n*-PrOH in *n*-hexane. Tlc monitoring of the fractionation process was carried out on Si gel 60–254 plates (E. Merck).

TWO-CHOICE, DIET-INCORPORATED ANTIFEEDANT BIOASSAY.—Pure compounds were dissolved in appropriate solvents and applied to powdered cellulose, which was then desiccated overnight to remove solvent. Cellulose-powder-containing sample was placed in a test tube (7.5 cm × 1.0 cm), and the tube was filled to the 4-cm mark with liquid artificial fall armyworm diet medium (Bioserv #F9179). Test tube contents were thoroughly mixed on a Vortex-Genie mixer. The diet-containing sample was drawn from the test tubes into a plastic soda straw (19.8 cm × 0.5 cm) to a height of 10 cm. Diet medium was allowed to gel, pushed out of the straws, and cut into 0.5-cm sections. Two of these sections containing the sample to be tested were placed opposite one another in a 5-cm plastic Petri dish. In the same dish, also placed opposite one another, were two sections of "control" diet that were prepared the same way, using the same solvent but containing no sample material. Each diet section was equidistant from the one next to it and approximately 0.5 cm in from the sides of the Petri dish. Each straw contained enough material to set up 10 Petri dishes; 5 replicates were prepared for each sample, and each sample was tested against fall armyworm larvae and European corn borer larvae. Twenty newly hatched fall armyworm larvae were placed in each of 5 Petri dishes, and 20 newly hatched European corn borer larvae were placed in each of the other 5 Petri dishes. Petri dishes containing insects were then placed in darkness at 27° for 16–20 h; following this the dishes and contents were frozen to terminate feeding, and the number of larvae at each diet type in each dish was recorded. Feeding ratios were then determined by dividing the total number of insects on the treated diet from all the replicates by the total number of insects on the control diet from all the replicates. Compounds **1** and **2** were tested at various levels, and the results are summarized in Table 3. Significant difference levels were determined by Chi-square analysis. Some samples were retested at certain levels, and the numbers from these tests were combined with those of the first test because they did not differ significantly from one another.

TOXICITY BIOASSAY.—Toxicity tests involved incorporating samples into the diet and drawing that mixture into soda straws, which were then cut into 0.5-cm sections (in the same manner as described for the antifeedant bioassy) and placed one per cup. Fall armyworm larvae or European corn borer larvae (15 per trial) were placed in the cups containing treated diets (one larva per cup). Compounds **1** and **2** were tested at concentrations of 1600, 800, 400, 200, 100, 50, and 25 ppm. Larval weights less than 5% of the controls, as measured on day 8, normally lead to 100% mortality before pupation. Thus both **1** and **2** were 100% effective against both species at 200 ppm and above.

SANDORICIN [**1**].—Compound **1** was obtained in 0.65% yield (based on weight of seed with seed coats removed) and crystallized from 10% *n*-PrOH in *n*-hexane, mp 215–217°; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; cims *m/z* (rel. int.) 589(88), 529(100); hreims found *m/z* $[M]^+$ 588.2570; $C_{31}H_{40}O_{11}$ requires 588.2576.

6-HYDROXYSANDORICIN [**2**].—Compound **2** was obtained in 0.45% yield (based on weight of seed with seed coats removed) and crystallized from 10% *n*-PrOH in *n*-hexane. Mp 250–255° (dec); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; cims *m/z* (rel. int.) 605(98), 587(8), 545(100), 485(10); hreims found *m/z* $[M]^+$ 604.2525; $C_{31}H_{40}O_{12}$ requires 604.2473.

X-RAY DATA.[3]—*Sandoricin* [**1**].—A single crystal of **1** ($C_{31}H_{40}O_{11}$) grown from an *n*-PrOH/hexane solution was used for a single crystal X-ray diffraction analysis. The crystal data were: size ca. 0.15 × 0.5 × 0.2 mm; orthorhombic; space group $P2_12_12_1$ (Z = 8); cell dimensions a = 14.301(2),

[3]Atomic coordinates for these structures have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

$b = 17.464(3)$, $c = 24.123(4)$ Å; $V = 6021.3(16)$ Å^3; D_c 1.299 g/cm^3. The 4216 unique reflections were collected by ω scans within $2\theta \leq 110°$ on a Nicolet $P2_1$ diffractometer using graphite monochromated CuKα radiation ($\lambda = 1.5418$ Å) radiation. All the nonhydrogen atomic positions were found by an initial direct method (SHELXTL) and tangent formula recycling technique. Hydrogen atoms were included at calculated positions following partial least-squares refinements. Full-matrix least-squares refinements with anisotropic nonhydrogen atoms and fixed, isotropic, riding hydrogens converged to a standard R value of 0.050 for the 3603 reflections with $|F_o| \geq 4\sigma(|F_o|)$. A perspective view of the final X-ray model of sandoricin is given in Figure 1. It is important to note that the X-ray analysis entailed two independent molecules for sandoricin [**1**], but because they were identical within experimental error, only one is shown in the X-ray figure.

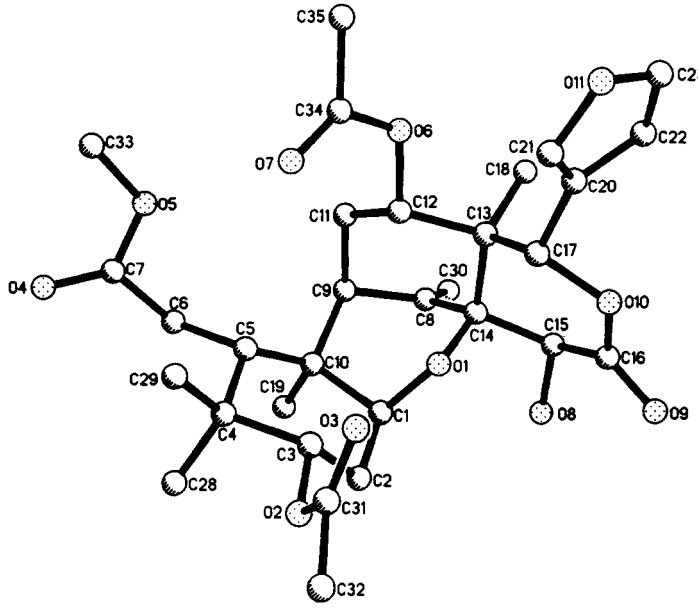

FIGURE 1. Computer-generated perspective drawing of the final X-ray model of sandoricin [**1**]. Hydrogens were omitted for clarity, and the absolute configuration shown was assumed.

6-Hydroxysandoricin [**2**].—A single crystal of **2** ($C_{31}H_{40}O_{12}$) grown from an *n*-PrOH/hexane solution was used for a single crystal X-ray diffraction analysis. The crystal data were: size ca. $0.1 \times 0.3 \times 0.5$ mm; triclinic; space group *P1* ($Z = 1$); cell dimensions $a = 8.867(3)$, $b = 9.707(3)$, $c = 9.990(3)$ Å, $\alpha = 104.75(2)$, $\beta = 105.24(2)$, $\gamma = 101.61(2)°$; $V = 768.4(4)$ Å^3; D_c 1.307 g/cm^3. The 2079 unique reflections were collected by 2θ–θ scans within $2\theta \leq 110°$ on a Nicolet $P2_1$ diffractometer using graphite monochromated CuKα radiation (1.54178 Å) radiation. All the nonhydrogen atomic positions were found by an initial direct method (SHELXTL) and tangent formula recycling technique. Following partial least-squares refinements, hydrogen atoms were included at calculated positions. Full-matrix least-squares refinements with anisotropic nonhydrogen atoms and fixed, isotropic, riding hydrogens converged to a standard R value of 0.044 for 2027 reflections with $|F_o| \geq 4\sigma(|F_o|)$. A perspective view of the final X-ray model of 6-hydroxysandoricin is given in Figure 2.

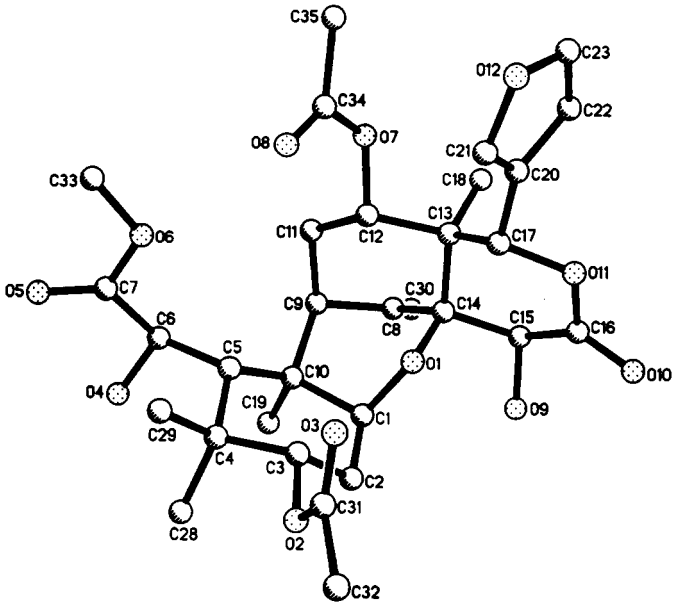

FIGURE 2. Computer-generated perspective drawing of the final X-ray model of 6-hydroxysandoricin [**2**]. Hydrogens were omitted for clarity, and the absolute configuration shown was assumed.

ACKNOWLEDGMENTS

We thank the National Institute of Health (CA24487) for financial support, Dr. D. Weisleder for nmr spectra, R.D. Plattner for mass spectra, and Dr. R.J. Bartelt for developing the bioassay and statistical analysis procedures.

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Received 6 *August* 1990