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HYDROXYALK-(4Z)-ENOIC ACIDS AND VOLATILE COMPONENTS  
FROM THE SEEDS OF *ZANTHOXYLUM ARMATUM*<sup>1</sup>

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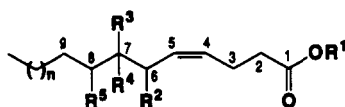
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**ABSTRACT.**—Nonpolar constituents of the hexane extract of the seeds of *Zanthoxylum armatum* (Rutaceae) have been identified as 6-hydroxynonadec-(4Z)-enoic acid [1], 8-hydroxypentadec-(4Z)-enoic acid [2], 7-hydroxy-7-vinylhexadec-(4Z)-enoic acid [3], and hexadec-(4Z)-enoic acid [4]. In addition, the essential oil has been found to contain mainly linalool (58.3%), limonene (24.46%), and methyl cinnamate (8.92%), besides several minor constituents.

*Zanthoxylum armatum* DC. (syn. *Zanthoxylum alatum* Roxb.) (Rutaceae) is an erect shrub or a small tree commonly occurring in hot valleys of the Himalayas. While its bark has been reported to possess active alkaloids, the fruits and seeds are also extensively used in the indigenous system of medicine (1). The fruits have been reported to yield an essential oil with linalool as the major constituent (2). The seeds have, earlier, been found to contain *cis*-9-hexadecenoic (3), eicosenoic, and palmitic acids (4) along with tambuletin (5). The present paper deals with the first detailed investigation of non-polar constituents of the seeds.

## RESULTS AND DISCUSSION

The hexane extract of the seeds, on chromatographic separations, yielded a complex mixture of straight chain compounds. On rigorous purification, four racemic alkenoic acids were obtained whose structures were determined mainly by spectroscopic methods. Compound 1 showed  $[M]^+$  at  $m/z$  312.2665 in its hrms spectrum suggesting the formula  $C_{19}H_{36}O_3$  (calcd 312.2664). It also showed typical fragments for a straight chain hydrocarbon until  $C_{13}H_{27}$ . The ions at  $m/z$  45, 60, and 73 clearly suggested that the chain ends with the  $-CH_2CH_2COOH$  unit. The fragments at  $m/z$  73 and 99 indicated that the molecule is a 4,5-enoic acid. Its  $^1H$ -nmr spectrum showed a broad triplet ( $J = 6.0$  Hz) at  $\delta$  0.88 and a broad singlet at  $\delta$  1.25 typical for a straight chain hydrocarbon; also olefinic methine signals at  $\delta$  5.26 as a broad doublet ( $J = 11$  Hz) of triplets



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	n
<b>1</b>	H	OH	H	H	H	9
<b>1a</b>	Me	OH	H	H	H	9
<b>1b</b>	H	OAc	H	H	H	9
<b>2</b>	H	H	H	H	OH	5
<b>2a</b>	Me	H	H	H	OH	5
<b>2b</b>	H	H	H	H	OAc	5
<b>3</b>	H	H	OH	CH=CH <sub>2</sub>	H	6
<b>3a</b>	Me	H	OH	CH=CH <sub>2</sub>	H	6
<b>3b</b>	H	H	OAc	CH=CH <sub>2</sub>	H	6
<b>4</b>	H	H	H	H	H	6
<b>4a</b>	Me	H	H	H	H	6

<sup>1</sup>CIMAP approval number 1092.

( $J = 5.5$  Hz) were indicative of a *cis* double bond. In addition, a multiplet at  $\delta$  3.60 suggested that **1** is a hydroxyalk-(4Z)-enoic acid. When treated with  $\text{CH}_2\text{N}_2$ , **1** also gave its methyl ester, confirming it to be an acid. The  $^{13}\text{C}$ -nmr spectrum showed a comparatively deshielded signal at  $\delta$  70.0, indicating that the OH group is attached to a carbon next to the double bond. Location of the OH group at C-6 was deduced by the significant  $\alpha$ -fission ions at  $m/z$  99, 129, 183, and 213. The rest of the  $^{13}\text{C}$ -nmr values (Table 1), as well as its ir spectrum, established that **1** is 6-hydroxynonadec-(4Z)-enoic acid.

TABLE 1.  $^{13}\text{C}$ -nmr Data for Compounds 1-4.

Carbon	Compound			
	1	2	3	4
C-1 . . . . .	174.2	178.0	173.3	180.8
C-2 . . . . .	34.3	34.7	34.5	34.3
C-3 . . . . .	27.5	27.8	27.5	27.3
C-4 . . . . .	130.0	130.2	130.0	130.0
C-5 . . . . .	128.0	128.2	128.2	128.2
C-6 . . . . .	70.0	25.5	45.3	25.0
C-7 . . . . .	34.2	32.5	73.0	29.8 <sup>a</sup>
C-8 . . . . .	29.8 <sup>a</sup>	65.5	32.0	29.8 <sup>a</sup>
C-9 . . . . .	29.8 <sup>a</sup>	30.0 <sup>a</sup>	29.9 <sup>a</sup>	29.5 <sup>a</sup>
C-10 . . . . .	29.5 <sup>a</sup>	29.9 <sup>a</sup>	29.9 <sup>a</sup>	29.5 <sup>a</sup>
C-11 . . . . .	29.5 <sup>a</sup>	29.9 <sup>a</sup>	29.9 <sup>a</sup>	29.1 <sup>a</sup>
C-12 . . . . .	29.5 <sup>a</sup>	29.5 <sup>a</sup>	29.8 <sup>a</sup>	29.0 <sup>a</sup>
C-13 . . . . .	29.2 <sup>a</sup>	32.5 <sup>a</sup>	29.5 <sup>a</sup>	32.2 <sup>a</sup>
C-14 . . . . .	29.2 <sup>a</sup>	23.1	32.0	22.2
C-15 . . . . .	29.1 <sup>a</sup>	14.3	23.0	14.0
C-16 . . . . .	29.0	—	14.0	—
C-17 . . . . .	32.0	—	145.0	—
C-18 . . . . .	23.0	—	112.2	—
C-19 . . . . .	14.2	—	—	—

<sup>a</sup>Assignments may be interchanged.

Compound **2**, on the other hand, showed  $[\text{M}]^+$  at  $m/z$  256.2039 in its hrms spectrum, indicating the possibility of its molecular formula being  $\text{C}_{15}\text{H}_{28}\text{O}_3$  (calcd 256.2038). The typical fragments at  $m/z$  45, 60, 73, and 99 indicated that **2** is also an alk-(4Z)-enoic acid analogous to **1**. The typical fragments for a straight chain hydrocarbon were visible until  $\text{C}_7\text{H}_{15}$  indicating that substitution may have occurred at C-8. The  $^1\text{H}$ -nmr values were similar to those of **1**, while  $^{13}\text{C}$  nmr clearly differed, with the signal at  $\delta$  65.5 (Table 1) suggesting that the OH group is not attached to C-6. The prominent fragments at  $m/z$  99, 157, as well as 129 and 127, clearly showed that the OH group is attached at C-8. These data along with the ir values confirmed that **2** is 8-hydroxypentadec-(4Z)-enoic acid.

Compound **3**, in its hrms showed  $[\text{M}]^+$  at  $m/z$  296.2350 suggesting the possibility of the molecular formula  $\text{C}_{18}\text{H}_{32}\text{O}_3$  (calcd 296.2351). The molecule also showed fragments typical for an alk-(4Z)-enoic acid, as in the case of **1** and **2**. The fragments until  $\text{C}_9\text{H}_{19}$  were clearly visible in the spectrum suggesting the possibility of substitution beyond this carbon. The  $^1\text{H}$ -nmr spectrum showed the double triplets ( $J = 11, 6.5$  Hz) at  $\delta$  5.55 for the *Z*-olefinic protons. In addition, the presence of the typical signals [ $\delta$  5.88 (dd,  $J = 9, 16$  Hz), 4.97 (d,  $J = 9$  Hz), 5.15 (d,  $J = 16$  Hz)] for the isolated

vinyl group and preparation of the acetate derivative suggested a substitution of HO-C-CH=CH<sub>2</sub>. The deshielded values of the methine proton in the <sup>1</sup>H-nmr spectrum could be attributed to this substitution being in its close vicinity, but the signal at δ 45.3 in the <sup>13</sup>C nmr clearly showed that this substitution had occurred at C-7 leaving C-6 in between this substitution and the olefinic bond. The ir values and other <sup>13</sup>C-nmr signals (Table 1) established that **3** is 7-hydroxy-7-vinylhexadec-(4Z)-enoic acid.

Compound **4** showed [M]<sup>+</sup> at *m/z* 254.2248 in its hrms for the possibility of a molecular formula C<sub>16</sub>H<sub>30</sub>O<sub>2</sub> (calcd 254.2246). It also showed a fragmentation pattern similar to those of compounds **1–3** indicative of an alk-(4Z)-enoic acid. Since its <sup>1</sup>H-nmr and <sup>13</sup>C-nmr spectra did not show any signal corresponding to further substitution (Table 1) in the molecule, it was obviously deduced for the structure of hexadec-(4Z)-enoic acid. The other spectral values were in complete agreement with the above structure (see Experimental).

The essential oil of this species has also been found to possess ascaricidal, antibacterial, anthelmintic, and antifungal properties (6–9). Therefore, we included this oil in our screening program (10–12) and investigated it in detail by using glc and gc-ms. The fragrant essential oil, obtained after hydrodistillation of the seeds (yield 3.5%), was chromatographed to obtain linalool, limonene, and methyl cinnamate in pure form. The glc and gc-ms analysis showed 27 well-resolved peaks, including six monoterpene hydrocarbons (28.5%), seven oxygenated monoterpenes (57.15%), one phenyl propanoid (8.82%), two sesquiterpene hydrocarbons (1.48%), and a sesquiterpene alcohol (0.21%) along with a few which were unidentified (3.83%). The minor constituents were mainly identified by ms fragmentation patterns (13), while the major components were identified by their ir and nmr spectra. The presence of linalool in such a high concentration (55.3%) makes the oil highly fragrant and attractive. Most of the essential-oil-bearing plants with linalool and linalyl acetate as major constituents have acquired important places for their commercial cultivation (14), and it is herewith suggested that the seeds of *Z. armatum* may also be commercialized as a rich source of linalool.

## EXPERIMENTAL

**PLANT MATERIAL.**—The fruits of *Z. armatum* were collected in October 1984 from Munshiyari of the district Pithoragarh of Uttar Pradesh Hills of the Central Himalayas, while the seeds were purchased from the local market. The voucher specimen was deposited in the herbarium of our institute. Dr. N. C. Shah, Head of the Botany Division, identified the material. Mp's are uncorrected; ir spectra were recorded in KBr pellets and CHCl<sub>3</sub>; 90 MHz <sup>1</sup>H-nmr and 20 MHz <sup>13</sup>C-nmr spectra were measured in CDCl<sub>3</sub> with TMS as internal standard.

**EXTRACTION AND FRACTIONATION OF SEEDS.**—Seeds (5 kg), after extraction with *n*-hexane, gave 200 g of extract which after cc over Si gel gave the following 11 fractions: fractions 1–3 (hexane), fraction 4 [hexane-EtOAc (19:1)], fraction 5 [hexane-EtOAc (9:1)], fraction 6 [hexane-EtOAc (4:1)], fractions 7 and 8 [hexane-EtOAc (7:3)], fraction 9 [hexane-EtOAc (1:1)], fraction 10 [hexane-EtOAc (3:7)], and fraction 11 (EtOAc). Fractions 2, 6, and 7 were further subjected to chromatographic separations. Fraction 2, after further cc over Si gel, yielded **4** [560 mg, *R<sub>f</sub>* 0.28, hexane-EtOAc (9:1)], while fraction 6 gave **3** [230 mg, *R<sub>f</sub>* 0.30, hexane-EtOAc (19:1)]. Fraction 7, after further cc (Si gel, hexane/EtOAc) and tlc [CH<sub>2</sub>Cl<sub>2</sub>-MeOH (19:1)] yielded **1** (80 mg, *R<sub>f</sub>* 0.38) and **2** (70 mg, *R<sub>f</sub>* 0.30).

**EXTRACTION AND ANALYSIS OF ESSENTIAL OIL FROM FRUITS.**—The fruits (2 kg) were hydrodistilled to yield 70 ml of oil with *d*<sup>36°</sup> 0.9321, *n*<sup>25</sup><sub>D</sub> 1.4780, [α]<sup>29</sup><sub>D</sub> +0.065°, acid value 3.92, ester value 24.31, ester value after acetylation 50.49. The major constituents of the oil were separated by column (Si gel, petroleum ether/C<sub>6</sub>H<sub>6</sub>) and tlc [Si gel, petroleum ether-C<sub>6</sub>H<sub>6</sub> (9:1)] chromatographic separations. The gc-ms of the essential oil was carried out as reported earlier (12), which showed the presence of linalool (55.30%), limonene (22.46%), methyl cinnamate (8.82%), and myrcene (3.55%) as major constituents and α-thujene (1.65%), 1,8-cineole (0.25%), *p*-cymene (0.65%), *cis*-ocimene (0.12%), γ-terpinene (0.08%), camphor (0.25%), α-fenchol (0.68%), carvone (0.20%), α-terpineol (0.31%), tagetonol

(0.16%),  $\beta$ -caryophyllene (0.50%), *allo*-aromadendrene (0.98%), and a sesquiterpene alcohol,  $[M]^+$  220 (0.21%).

**6-Hydroxynonadec-(4Z)-enoic acid [1].**—Mp 40–42°, ir  $\nu$  max  $\text{cm}^{-1}$  3600–3100 (OH, COOH), 2920–2840, 1725 (CO), 1458, 1230, 1170, 1100, 1040;  $^1\text{H}$  nmr 5.26 (overlapping dt,  $J = 11, 5.5$  Hz, H-4 and -5); 0.88 (tbr,  $J = 6.0$  Hz, H-19), 1.25 (sbr, H-8–H-18), 3.60 (m, H-6), 1.60–2.35 (overlapping m, H-2, -3, -7); hrms  $m/z$  (rel. int.)  $[M]^+$  312.2665 ( $\text{C}_{19}\text{H}_{36}\text{O}_3$ ) (1),  $[M - \text{H}_2\text{O}]^+$  294 (4), 239 (14), 213 (4), 183 (4), 169 (4), 155 (12), 143 (16), 141 (20), 129 (16), 127 (20), 113 (30), 99 (30), 85 (30), 73 (35), 71 (70),  $[\text{C}_2\text{H}_4\text{O}_2]^+$  60 (24), 57 (85), 45 (18), 43 (100). Compound **1** (10 mg) was taken in a flask, and a solution of  $\text{CH}_2\text{N}_2$  in  $\text{Et}_2\text{O}$  was added at room temperature. After usual work up **1a** was obtained (10 mg).  $^1\text{H}$  nmr gave an additional signal at  $\delta$  3.65. Compound **1** (20 mg) was refluxed with  $\text{Ac}_2\text{O}$  at 80° for 4 h. After usual work up **1b** was obtained (15 mg).  $^1\text{H}$  nmr gave an additional singlet at 2.05 along with a multiplet at 4.20.

**8-Hydroxypentadec-(4Z)-enoic acid [2].**—Viscous liquid: ir  $\nu$  max  $\text{cm}^{-1}$  3600–3100 (OH, COOH), 2920–2850, 1710 (CO), 1450, 1378, 1230, 1170;  $^1\text{H}$  nmr 5.30 (overlapping dt,  $J = 11, 5.5$  Hz, H-4 and H-5), 0.88 (t,  $J = 6.0$  Hz, H-15), 3.50 (m, H-8), 1.70–2.75 (overlapping m, H-2, -3, -6), 1.26 (sbr, H-9–H-14); hrms  $m/z$  (rel. int.)  $[M]^+$  256.2039 ( $\text{C}_{15}\text{H}_{28}\text{O}_3$ ) (1),  $[M - \text{H}_2\text{O}]^+$  238 (1), 183 (4), 171 (4), 157 (3), 143 (5), 129 (5), 127 (10), 113 (12), 99 (26), 85 (15), 73 (30), 71 (90),  $[\text{C}_2\text{H}_4\text{O}_2]^+$  60 (24), 57 (36), 45 (15), 43 (100). Compound **2** (10 mg) was treated with  $\text{CH}_2\text{N}_2$  as in case of **1** and **2a** was obtained (10 mg).  $^1\text{H}$  nmr showed a signal at  $\delta$  3.65 for OMe. After workup as in case of **1**, **2b** was obtained showing characteristic signals (2.08, 4.15) for its acetate derivative in the  $^1\text{H}$  nmr spectrum.

**7-Hydroxy-7-vinylhexadec-(4Z)-enoic acid [3].**—Viscous liquid: ir  $\nu$  max  $\text{cm}^{-1}$  3600–3100 (OH, COOH), 2920–2840, 1725 (CO), 1460, 1230, 1170;  $^1\text{H}$  nmr 5.50–5.60 (overlapping dt,  $J = 11, 5.5$  Hz, H-4 and -5), 0.85 (tbr,  $J = 6.0$  Hz, H-16), 1.25 (sbr, H-8–H-15), 3.43 (s, OH), 1.95–2.30 (overlapping multiplets, H-2, -3, and -6), 5.88 (dd,  $J = 9$  and 16 Hz, H-17), 4.97 (dbr,  $J = 9$  Hz, H<sub>a</sub>-18), 5.15 (dbr,  $J = 16$  Hz, H<sub>b</sub>-18); hrms  $m/z$  (rel. int.)  $[M]^+$  296.2350 ( $\text{C}_{18}\text{H}_{32}\text{O}_3$ ) (0.1),  $[M - \text{H}_2\text{O}]^+$  278 (2.5), 236 (4), 223 (3), 197 (3), 183 (4), 169 (5), 127 (12), 113 (10), 99 (15), 85 (20), 73 (12), 71 (78),  $[\text{C}_2\text{H}_4\text{O}_2]^+$  60 (15), 57 (45), 45 (100), 43 (97). Compound **3** (10 mg) was methylated as in the case of **1** and a typical MeO signal ( $\delta$  3.66) in the  $^1\text{H}$  nmr was observed for **3a**. Compound **3** was reacted with pyridine and  $\text{Ac}_2\text{O}$  for 24 h at room temperature. After usual workup **3b** was obtained having the characteristic signal ( $\delta$  2.10) for the acetate.

**Hexadec-(4Z)-enoic acid [4].**—Mp 49–50; ir  $\nu$  max  $\text{cm}^{-1}$  3400–3100 (COOH), 2900–2840, 1710 (CO), 1460, 1290;  $^1\text{H}$  nmr 5.30 (overlapping dt,  $J = 11.0, 5.5$  Hz, H-4 and -5), 0.87 (t,  $J = 6.0$  Hz, H-15), 2.45–1.50 (overlapping m, H-2, -3, and -6), 1.26 (s, br, H-7–H-14); hrms  $m/z$  (rel. int.)  $[M]^+$  254.2248 ( $\text{C}_{16}\text{H}_{30}\text{O}_2$ ) (100), 194 (10), 181 (3), 155 (5), 141 (8), 113 (12), 99 (14), 85 (32), 73 (78), 71 (42),  $[\text{C}_2\text{H}_4\text{O}_2]^+$  60 (63), 57 (63), 45 (8), 43 (68). Compound **4** (50 mg) was methylated with  $\text{CH}_2\text{N}_2$  as in the case of **1**, and the product was verified by  $^1\text{H}$  nmr ( $\delta$  3.64 for the MeO).

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