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7-DEACETYL-17β-HYDROXYAZADIRADIONE, A NEW LIMONOID INSECT GROWTH INHIBITOR FROM *AZADIRACHTA INDICA*

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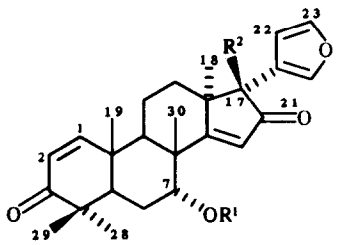
Key Word Index—*Azadirachta indica*; limonoids; tetranortriterpenoids; 7-deacetyl-17β-hydroxyazadiradione; azadiradione; 7-deacetylazadiradione; *Heliothis virescens*; insect growth inhibitor.

Abstract—A new tetranortriterpenoid of the limonoid type, 7-deacetyl-17β-hydroxyazadiradione, and the known compound azadiradione, were isolated from the seeds of *Azadirachta indica*. The structure of the new compound was established by spectroscopic methods. The activity of the new compound as an insect growth inhibitor against *Heliothis virescens* was found to be greater than that of azadiradione and 7-deacetylazadiradione.

INTRODUCTION

Azadirachta indica A. Juss. (Meliaceae), the neem tree, is widely distributed in dry areas of the tropics and subtropics [1]. A number of triterpenoids have been isolated and identified from neem [2]. Some of these compounds have been shown to be biologically active, especially against insects [3–5].

Herein we report on the isolation and characterization of a new tetranortriterpenoid of the limonoid type, 7-deacetyl-17β-hydroxyazadiradione(**1**), together with the known parent compound azadiradione(**2**), from neem seeds. The insect growth inhibitory activity of these compounds, and that of the known compound 7-deacetylazadiradione(**3**) against *Heliothis virescens* (Fabr.) (tobacco budworm) is now reported.



1 R¹ = H, R² = OH
2 R¹ = Ac, R² = H
3 R¹ = H, R² = H
4 R¹ = Ac, R² = OH

RESULTS AND DISCUSSION

Compound **1** was isolated from the dichloromethane extract of the neem seeds using insect bioassay-guided fractionation involving reverse phase HPLC. The IR spectrum of compound **1** indicates the presence of hydroxyl groups (3450 cm^{-1}), and cyclopentenone (1710 cm^{-1}), cyclohexenone (1670 cm^{-1}), and furan (880 cm^{-1}) ring moieties. The proton-decoupled ^{13}C NMR spectrum of **1** indicates the presence of five Me, three CH_2, nine CH, and nine 'quaternary' carbons using DEPT spectral editing procedures [6]. These data, together with the molecular ion peak at *m/z* 424 in the mass spectrum of compound **1**, suggest its molecular formula to be $C_{26}H_{32}O_5$. The 11 double bond equivalents indicated in the molecular formula can be accounted for by a furan ring (3 equivalents), two α,β-unsaturated ketone systems (4 equivalents), and the remaining four equivalents by the four rings of the tetracyclic triterpenoid nucleus.

The ^1H NMR spectrum of **1** shows singlets at δ0.98, 1.11, 1.17, 1.22, and 1.30, indicative of five tertiary methyl groups. The H-1 and H-2 protons display two doublets of the AB type centered at δ7.14 and 5.86 (J = 10.3), respectively, characteristic of a ring-A 1-ene-3-one system. The H-21, H-22, and H-23 protons display multiplets at δ7.59, 6.41 and 7.43, respectively, characteristic of a β-substituted furan ring. These data, together with the IR and MS data, show a close structural similarity of compound **1** to azadiradione (**2**) [7], 7-deacetylazadiradione (**3**) [8], and 17β-hydroxyazadiradione (**4**) [9, 10]. We base our NMR assignments (Table 1) upon those reported for compound **4** [9], on chemical shifts and multiplicities found for compounds **1–3**, and on our 2D-NMR correlations for compound **1** mentioned below.

The lack of an acetyl group at C-7 in **1** was established by noting that a lower field methyl signal (δ1.92 in **2**) is absent in its ^1H NMR spectrum and that D_2O exchange produces loss of two signals, assigned to the OH protons at positions 7 (δ1.88) and 17 (δ2.66). In addition, the resonance at δ4.20, assigned to H-7, changes from a quartet-like multiplet (*ddd*) to an apparent triplet (scalar coupling to the methylene protons at C-6) after exchange of the 7-OH proton.

In their structural elucidation of 17β-hydroxyazadiradione (**4**), Kraus and Cramer [9] assigned the carbons at positions 6, 11, and 12, but did not differentiate the corresponding methylene protons. By establishing the

Table 1. Proton and ^{13}C NMR data* of 7-deacetyl-17β-hydroxyazadiradione

Position†	Proton	^{13}C
1	7.14 (1H, *d*, $^3J_{1,2}=10.3$)	157.49
2	5.86 (1H, *d*, $^3J_{2,1}=10.3$)	125.78
3	—	204.69
4	—	44.20
5	2.40 (1H, *m*)	36.95
6	1.89 (2H, *m*)	25.48
7	4.20 (1H, *ddd*, $J=2.7$)	71.78
	1.88 (1H, *d*, OH)	
8	—	46.84
9	2.52 (1H, *m*)	44.46
10	—	40.20
11	2.04 (2H, *m*)	15.67
12	1.62 (2H, *m*)	22.19
13	—	50.43
14	—	195.31
15	5.92 (1H, *s*)	120.23
16	—	206.25
17	2.66 (1H, *s*, OH)	80.78
18	0.98 (3H, *s*)	30.19
19	1.22 (3H, *s*)	19.20
20	—	122.43
21	7.59 (1H, *dd*)	141.45
22	6.41 (1H, *dd*)	109.54
23	7.43 (1H, *dd*)	142.56
28	1.11 (3H, *s*)	21.44
29	1.17 (3H, *s*)	27.13
30	1.30 (3H, *s*)	25.57

*Chemical shift (δ) in ppm from TMS.
†Structure **1**.

connectivities (scalar coupling interactions) between the previously assigned protonated carbons and the protons directly bonded to them, we were able to assign the methylene protons. For example, two of the three methylene proton–carbon interactions, namely C-12-H_2 and C-6-H_2, are evident as spots on the contour plot of the 2D C-H (HETCOR) measurements. The highest field proton multiplet, centered at δ1.62, is assigned to the C-12 methylenes (Table 1). Evidently, the 17-OH provides anisotropic shielding, since the methylene proton resonances in compounds **2** and **3** all occur in the δ1.8–2.1 region (Experimental).

The C-6 methylene protons are assigned to the next highest-field two-proton pattern at δ1.89 because of the strong correlation seen with H-7 in the homonuclear proton COSY spectrum. The C-11 methylene protons are thus assigned to δ2.04 by elimination.

In order to specify the stereochemistry at position 17, we used a proton nuclear Overhauser effect (NOESY) experiment which indicates dipolar through-space interactions between closely aligned protons [11]. Our experiment showed that H-21 and H-22 on the furan ring are close enough to interact with the C-18 methyl protons. Therefore, both the C-18 methyl and the furan must be on the same side of the triterpenoid nucleus, thereby establishing the beta stereochemistry at C-17 of compound **1**.

In order to compare the insect growth inhibitory activity of compound **1** with that of related compounds,

Table 2. Growth inhibitory activity of 7-deacetyl-17β-hydroxyazadiradione and two related limonoids fed in an artificial diet to first instar *Heliothis virescens*

Test compounds	*EC_{50} (ppm)	†Confidence limits
7-Deacetyl-17β-hydroxyazadiradione	240	105–550
Azadiradione	560	354–886
7-Deacetylazadiradione	1600	1293–1980

*EC_{50} is the effective concentration of additive necessary to reduce larval growth to 50% of the control values.
†Confidence limits were determined using the method of ref. [17].

we isolated azadiradione (**2**) [7] and bioassayed it and its alkaline hydrolysis product, 7-deacetylazadiradione (nimbocinol) (**3**) [8] in artificial diet against *H. virescens*. Compound **1**, which has hydroxyl groups at both the C-7 and C-17 positions, was *ca* 7-fold more active (EC_{50} = 240 ppm) than was **3** (EC_{50} = 1600 ppm), which has a hydroxyl group only at the C-7 position (Table 2). Compound **2**, which has no hydroxyl groups, was *ca* 3-fold more active (EC_{50} = 560 ppm) than was **3** (Table 2). Similarly, **2** was found to be more active than **3** as an insect growth inhibitor against larvae of the pink bollworm (*Pectinophora gossypiella*), the fall armyworm (*Spodoptera frugiperda*), and the corn earworm (*Heliothis zea*) [12].

Apparently, a hydroxyl group at C-7 reduces the insect growth inhibitory activity of azadiradione (**2**), while a hydroxyl group at C-17 increases the activity of 7-deacetylazadiradione (**3**), at least against *H. virescens*. Hydroxyl groups in other groups of limonoids were also found to influence biological activity. For example, acetylation or ketonization of the C-7 or C-12 hydroxyl groups in the trichilins rendered them inactive as antifeedants against larvae of the southern armyworm (*Spodoptera eridania*) [13]. On the other hand, deacetylation of the C-1 acetate group in nomilin rendered it inactive as a growth inhibitor against larvae of the fall armyworm and the corn earworm [12].

EXPERIMENTAL

Extraction, isolation and purification. Air-dried neem seeds were obtained from India by Vikwood, Ltd, Sheboygan, Wisconsin. The seeds were extracted with MeOH, defatted, and partitioned as previously described [14, 15]. The fraction partitioning into CH_2Cl_2 was used to isolate both compounds **1** and **2**.

Isolation of 7-deacetyl-17β-hydroxyazadiradione (**1**). A part of the CH_2Cl_2 fraction (*ca* 19 g) from the neem seeds was subjected to CC on C_{18} (40 μm, 10 × 17 cm). The column was eluted stepwise with increments of aq. MeOH in H_2O (55, 60, 65 and 70%). The latter two fractions were combined, reduced in vol., and subjected to CC on silica gel (40 μm, 2.5 × 43 cm). The column was eluted with Et_2O–CH_2Cl_2 (3:1) at 2 ml/min. The fraction eluting between 100 and 150 min was collected and subjected to reverse phase HPLC (phenyl, 5 μm, 25 × 2.25 cm i.d.) run isocratically with MeCN–H_2O (7:13) at a flow rate of 5.0 ml/min and an average pressure of 900 psi. This fraction yielded 36 mg of 7-deacetyl-17β-hydroxyazadiradione (**1**) (R_t = 9.9 min).

7-Deacetyl-17β-hydroxyazadiradione (**1**). Colourless needles (MeOH); IR γ_{max}^{KBr} cm^{-1}: 3450 (OH), 1710 (cyclopentenone), 1670 (cyclohexenone), 880 (furan); UV λ_{max}^{MeOH} nm: 238 and 219; FABMS (positive ion mode), *m/z*:425$[M+H]^+$ ($C_{26}H_{33}O_5$), corresponding to a molecular formula of $C_{26}H_{32}O_5$ (M 424); EIMS (direct probe), 70 eV, *m/z* (rel. int.): 424 $[M]^+$ (84), 409 (59), 406 (12), 391 (12), 190 (16), 189 (14), 149 (12), 137 (14), 135 (14), 121 (12), 95 (100).

Isolation and identification of azadiradione (**2**). Another part of the CH_2Cl_2 fraction (*ca* 15 g) was subjected to CC on Florisil (60–100 mesh, 5 × 115 cm). The column was eluted first with Et_2O, and then stepwise with 2.5 and 5% MeOH in Et_2O. Colourless needles separated from the latter fraction and they were recrystallized in MeOH (× 3) to yield 620 mg of azadiradione (**2**) [7].

Azadiradione (**2**). EIMS (direct probe), 70 eV, *m/z*:450$[M]^+$ ($C_{28}H_{34}O_5$); ^1H NMR (200 MHz, $CDCl_3$): δ7.44 (1H, *m*, H-21), 7.40 (1H, *m*, H-23), 7.10 (1H, *d*, $^3J_{1,2}=10.2$, H-1), 6.24 (1H, *m*, H-22), 5.86 (1H, *d*, $^3J_{2,1}=10.2$, H-2), 5.84 (1H, *s*, H-15), 5.29 (1H, *m*, H-7), 3.39 (1H, *s*, H-17), 2.46 (1H, *m*, H-9), 2.18 (1H, *m*, H-5), 2.10–1.79 (6H, *m*, H-6, H-11, H-12), 1.92 (3H, *s*, OAc), 1.31 (3H, *s*, 30-Me), 1.22 (3H, *s*, 19-Me), 1.06 (6H, *s*, 28-Me, 29-Me), 0.99 (3H, *s*, 18-Me).

Saponification of azadiradione and identification of 7-deacetylazadiradione (**3**). Azadiradione (**2**) (315 mg) was dissolved in 10 ml of 1% KOH in MeOH. The mixture was heated at 70° for 30 min, followed by extraction (× 3) into CH_2Cl_2. Colourless needles separated from the CH_2Cl_2 extract and they were recrystallized in MeOH (× 3) to yield 150 mg of 7-deacetylazadiradione (nimbocinol) (**3**) [8].

7-Deacetylazadiradione (nimbocinol) (**3**). FABMS (positive ion mode), *m/z*:409$[M+H]^+$ ($C_{26}H_{33}O_4$), corresponding to a molecular formula of $C_{26}H_{32}O_4$ (M 408); ^1H NMR (200 MHz, $CDCl_3$): δ7.48 (1H, *m*, H-21), 7.44 (1H, *m*, H-23), 7.13 (1H, *d*, $^3J_{1,2}=10.2$, H-1), 6.27 (1H, *m*, H-22), 6.03 (1H, *s*, H-15), 5.87 (1H, *d*, $^3J_{2,1}=10.2$, H-2), 4.20 (1H, *ddd*, $J_{7,6a}\approx J_{7,6b}\approx J_{7,OH}\approx 2.8$, H-7), 3.45 (1H, *s*, H-17), 2.57 (1H, *m*, H-9), 2.46 (1H, *m*, H-5), 2.10–1.80 (6H, *m*, H-6, H-11, H-12), 1.75 (1H, *m*, OH), 1.26 (3H, *s*, 30-Me), 1.23 (3H, *s*, 19-Me), 1.18 (3H, *s*) and 1.12 (3H, *s*, 28-Me, 29-Me), 1.04 (3H, *s*, 18-Me).

Bioassay. As previously described [16], an artificial diet bioassay with newly hatched (first instar) larvae of the tobacco budworm, *H. virescens* was used to determine the potency of 7-deacetyl-17β-hydroxyazadiradione (**1**), azadiradione (**2**), and 7-deacetylazadiradione (**3**). Potency was determined as the effective concentration (EC_{50}) of compound added to the diet necessary to cause a 50% reduction in weight. EC_{50} values were determined from log probit paper and analysed statistically by the method of ref. [17].

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