

**AZADIRACHTANIN, A NEW LIMONOID FROM THE LEAVES OF AZADIRACHTA
INDICA**

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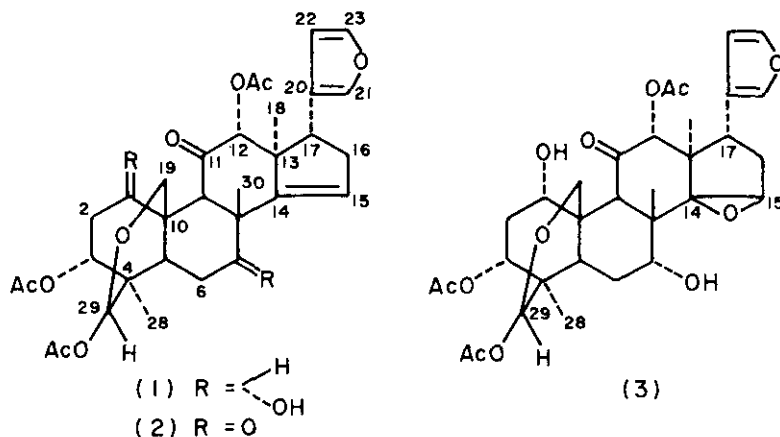
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Abstract - Azadirachtanin, a new limonoid of the Δ^{14} -meliacan skeleton has been isolated from the leaves of *Azadirachta indica* A. Juss and its structure has been deduced from its spectral data coupled with chemical evidence.

Azadirachta indica A. Juss (Syn. *Melia azadirachta* Linn), a well known species of the Meliaceae family grows wild in almost all the dry forests of India¹. The extensive uses² of the plant for the treatment of a variety of human diseases are reminiscent of broad spectrum activities of some modern medicines. A number of limonoids and other constituents have been isolated from the plant^{3,4}. We report herein the isolation and structure elucidation of a new limonoid, designated as azadirachtanin from the fresh leaves of the plant. Confirmation⁵ of hypoglycemic activity of EtOH extract of the fresh leaves prompted us to undertake a detailed chemical investigation of this extract.

Azadirachtanin (1), $C_{32}H_{40}O_{11}$, mp 225-226°C, $[\alpha]_D^{25} +8.7^\circ (CHCl_3)$ was isolated as colourless needles from the EtOH extract of the fresh leaves on fractionation with different organic solvents and repeated silica gel column chromatography. The uv spectrum did not show any characteristic absorption above 210 nm. The ir spectrum (KBr) showed absorptions at 3600, 3460, 3140, 1745, 1730, 1715, 1430, 1260, 1050, 910 and 880 cm^{-1} . The mass spectrum exhibited peaks at m/z : 600 (M^+), 585 ($M^+ - CH_3$), 582 ($M^+ - H_2O$), 540 ($M^+ - AcOH$), 480 ($M^+ - 2 \times AcOH$). These data along with 1H and ^{13}C nmr (Table I and Table II) suggested that (1) has a β -substituted furan ring, three acetate, two hydroxyl and one ketonic groups. Besides the two double bonds in the furan ring the presence of a third trisubstituted double bond was demonstrated by the ^{13}C nmr spectrum showing signals at δ 159.3(s) and 123.0(d). Moreover, 1H and ^{13}C nmr spectra of (1) showed characteristic peaks at δ 6.12 (1H, s), 4.57 (2H, s), 95.4(d) and 66.2 (t) attributable to a hemiacetal structure $-CH-O-CH_2-$. The presence of two secondary hydroxyl groups was confirmed by the oxidation of (1) with Jones' reagent which afforded a triketone (2), $C_{32}H_{36}O_{11}$, mp 205-207°C. The ^{13}C nmr spectrum of (1) assisted with off resonance technique revealed the presence of four quaternary carbons, three methines, four oxygen bearing



methines and three methylene carbons. The foregoing spectroscopic data suggested azadirachtanin to have a meliacan skeleton⁶.

Comparison of carbon chemical shifts of (1) with those of analogous carbon shifts of sendanin⁷ (3) revealed close agreement between the A and B ring carbons of the two compounds. The position and pattern of ¹H nmr signal assignable to the vinyl proton are reminiscent of those of Δ^{14} -meliacan derivative^{8,9}. Moreover, the trisubstituted double bond could be placed at 14, 15-position considering its effect on the neighbouring carbon atoms. In fact, the δ values of ring D carbons of (1) closely resembled those of sendanin⁹ containing Δ^{14} -double bond and a 12 α -acetoxy group. Thus on the basis of the evidences outlined above the structure of azadirachtanin is proposed as shown in (1).

It may be mentioned that complex representatives of the hemiacetal of 19-hydroxyhavanensin - 29-aldehyde (4) have been isolated from natural sources^{7,10,11} although this substance itself is still unknown. It appears that azadirachtanin (1) is the first member of the nucleus (5).

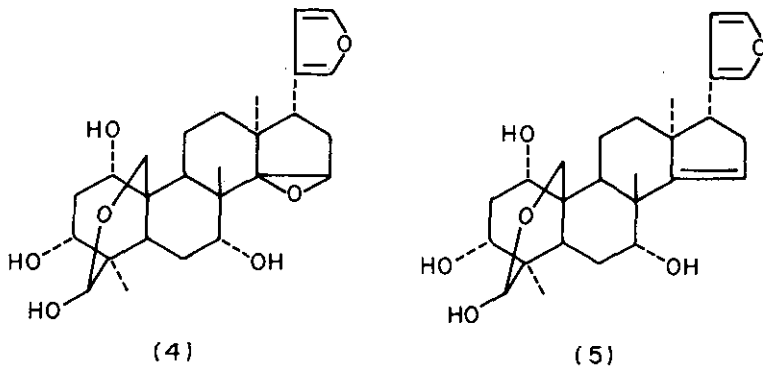


Table I. ^1H nmr spectra (100 MHz, CDCl_3)

(1)		(2)	
δ ppm	assignment	δ ppm	assignment
7.36(d,2)	H-23	7.32(d,2)	H-23
7.21(s)	H-21	7.15(s)	H-21
6.20(d,2)	H-22	6.10(d,2)	H-22
6.12(s)	H-29	6.04(s)	H-29
5.83(s)	H-12	5.84(t,2.5)	H-15
5.80(t,2.5)	H-15	5.64(s)	H-12
5.46(m,w _{1,2} 6)	H-3	5.50(m)	H-3
5.33(s)	H-9	4.60(br,s)	H-19
4.74(m)	H-1	4.16(s)	H-9
4.57(s)	H-19	1.90	3 x OAc
3.87(m)	H-7	1.86 (s,each)	
1.94	3 x OAc	1.82	
1.89 (s,each)		1.24(s)	H-18
1.86		1.10(s)	H-30
1.25(s)	H-18	0.76(s)	H-28
1.16(s)	H-30		
0.90(s)	H-28		

s = singlet, d = doublet, t = triplet, m = multiplet; coupling constants (Hz) are given in the parentheses.

Table II. ^{13}C nmr spectra of (1) and (3) (solvent CDCl_3 , δ values, TMS as standard)

Carbon atom	(1)	(3) ^d
1	70.2 ^a	70.1 ^a
2	36.6	36.6
3	74.1	74.0
4	39.8 ^b	39.8 ^b
5	29.1	29.0
6	26.6	26.5
7	71.2 ^a	69.5 ^a
8	43.2 ^b	42.2 ^b
9	49.4	49.8
10	43.3 ^b	43.2 ^b
11	208.8	208.7
12	79.0	79.1
13	50.8 ^b	46.5 ^b
14	159.3	73.2
15	123.0	59.1
16	36.3	34.2
17	50.1	39.2
18	19.9 ^c	20.7 ^c
19	66.2	66.0
20	123.8	124.0
21	142.0	143.2
22	111.3	112.8
23	140.5	141.6
28	16.2 ^c	19.2 ^c
29	95.4	95.6
30	18.6 ^c	15.7 ^c
	170.1	170.9 x 2
<u>COCH₃</u>	170.6	170.0
	170.9	
	22.7	22.8
<u>COCH₃</u>	21.3	21.1
	20.8	20.7

a - c may be interchanged in each vertical column.
d Reference 7

EXPERIMENTAL

Melting points are uncorrected. Uv spectra were recorded on a Specord UV - vis instrument. Ir spectra were taken on a Perkin-Elmer-Infracord Model 177. ^1H and ^{13}C nmr spectra were taken in CDCl_3 solution on a JEOL-FX-100 spectrometer at 100 and 25.15 MHz respectively with Me_4Si as internal reference. Mass spectra were obtained on Hitachi RMU-6L and MS-50 mass spectrometers at an ionizing potential of 70 eV.

Isolation of Azadirachtanin

The fresh leaves of *azadirachta indica* were collected locally in the month of October. A voucher specimen (IICB-472) was deposited in the herbarium of IICB. The fresh leaves (3 kg, water content 70%) were cut into pieces and extracted with EtOH at room temperature and the extract was concentrated under reduced pressure to a thick syrup which was then adsorbed on paper pulp, dried and extracted successively with light petroleum, chloroform and methanol. The methanol extract was concentrated under reduced pressure to yield a viscous dark brown mass (60 g). This was chromatographed on silica gel (950 g). Graded elution was effected with CHCl_3 and CHCl_3 -MeOH mixtures (98 : 2) and (95 : 5). A total of 90 fractions of 150 ml each were collected and mixed on the basis of t.l.c.

Azadirachtanin (1)

Fractions 46 - 65 eluted with CHCl_3 -MeOH (98 : 2) were mixed, purified by rechromatography and crystallised from Et_2O -MeOH mixture to give colourless needles of (1) (56 mg), mp 225 - 226°C, $[\alpha]_D + 8.7^\circ$ (c, 0.45 in CHCl_3). ^1H and ^{13}C nmr data are given in Table - I and Table - II respectively. Anal. Calcd for $\text{C}_{32}\text{H}_{40}\text{O}_{11}$: C, 63.98; H, 6.71. Found : C, 63.92; H, 6.75.

Oxidation of (1) to (2)

To a solution of (1) (15 mg) in acetone (3 ml) was added Jones' reagent (prepared by dissolving 2.6 g of Cr_2O_3 in 2.3 ml of concentrated H_2SO_4 and adding water to 10 ml total volume) drop by drop at 0°C while stirring until a permanent orange colour persisted. The stirring was continued for 15 minutes. Usual workup yielded the ketone (2) which crystallised from Et_2O -MeOH as flakes (11 mg), mp 205 - 207°C, $[\alpha]_D + 18.6^\circ$ (c, 1.25 in CHCl_3). ^1H nmr data are given in Table - I. Anal. Calcd for $\text{C}_{32}\text{H}_{36}\text{O}_{11}$: C, 64.42; H, 6.08. Found : C, 64.37; H, 6.12.

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