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STRUCTURE OF DEACETYLAZADIRACHTINOL
APPLICATION OF 2D ^{1}H-^{1}H AND ^{1}H-^{13}C SHIFT CORRELATION SPECTROSCOPY

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Abstract: A new insect antifeedant, and ecdysis inhibitor deacetylazadirachtinol, was isolated from Azadirachta indica (Meliaceae) collected in Haiti and its structure (1) was elucidated by the spectral comparison with azadirachtin using two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C shift correlation spectroscopies.

Azadirachta indica (Meliaceae), Indian Neem tree, has long been used for the control of various insect pests in Asia and other countries[1]. A potent insect antifeedant and ecdysis inhibitor[2] azadirachtin was isolated from the seeds of A. indica collected in Kenya and the structure was proposed to be a complex limonoid (2) mainly based on spectroscopic data[3]. In the search for new insect control agents there is much interest in the synthesis of azadirachtin. However the structure of azadirachtin (2) is too complicated for such a task to be economically feasible. Therefore, in order to accumulate structure-activity relationship data based on natural limonoids to identify the minimum necessary structural components for activity we have investigated the seed oil of A. indica collected in Haiti. Monitoring by both a leaf disk bioassay[4] and an artificial diet feeding assay[5], we have isolated the new congener (1): mp 148 °C, CD (MeOH) $\Delta\varepsilon$ (237 nm) -4.6, UV (EtOH) 222 nm (ε: 20000), IR ($CHCl_3$) 3440, 1730, 1720, 1700, 1690 and 1650 cm^{-1}, in addition to the known limonoids, salannin, 3-desacetylsalannin, 6-O-acetylnimbandiol and azadirachtin. We report herein the structural analysis of this new limonoid (1) utilizing by combination of the two-dimensional (2D) homo (^{1}H-^{1}H)- and heteto (^{1}H-^{13}C)-nuclear correlation spectroscopies (COSY)[6].

The SIMS m/z 645 $[M-2H_2O+H]^+$, the ^{1}H NMR (44 protons) and the ^{13}C NMR (33 carbons) indicated the molecular formula $C_{33}H_{44}O_{15}$. As can be seen in Table 1, the ^{1}H NMR and the ^{13}C NMR suggest that 1 is closely related to azadirachtin (2)

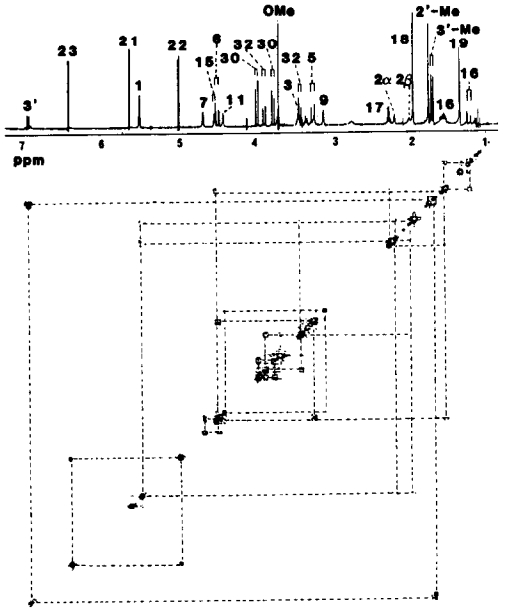
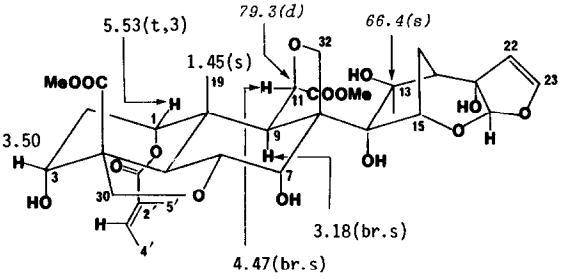
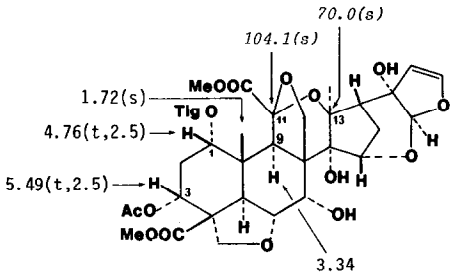


Figure 1. ^{1}H-^{1}H shift correlation of deacetlyazadirachtinol (1).



1

deacetylazadirachtinol



2

azadirachtin

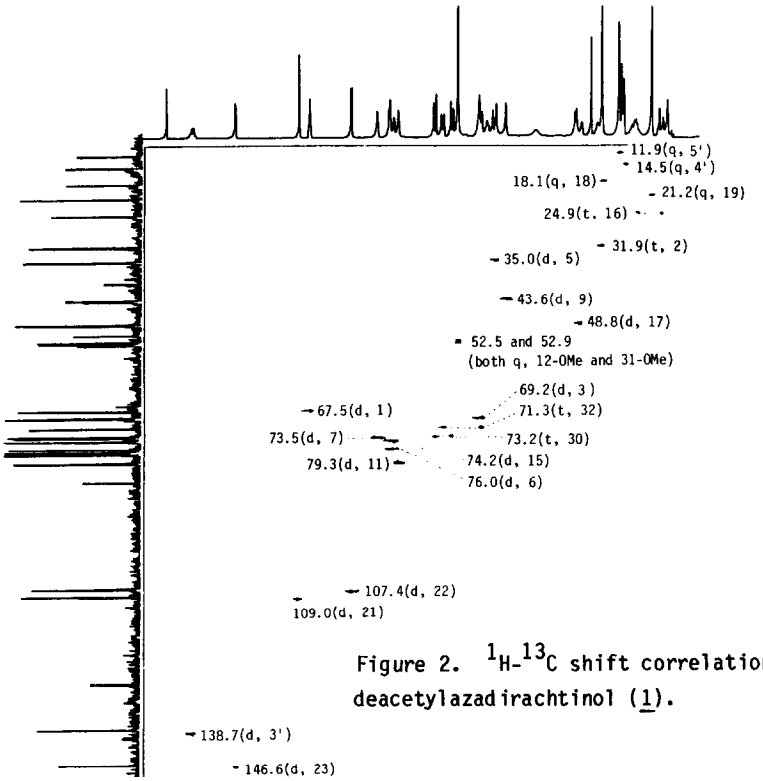


Figure 2. ^{1}H-^{13}C shift correlation of deacetylazadirachtinol (1).

except for the following points.

(i) In the ^{13}C NMR of 1, the signal corresponding to the ketal carbon (C11, 104.1 ppm) in azadirachtin (2) was not observed. Instead a tertiary carbon signal appeared at 79.3 ppm. This signal could be identified as C11 since the 4.47 ppm ^{1}H NMR signal of 1, coupling with the 79.3 ppm ^{13}C NMR signal in the ^{1}H-^{13}C 2D NMR spectrum (Figure 2), showed small vicinal coupling with the C9 proton signal (3.18 ppm) in the ^{1}H-^{1}H 2D NMR spectrum (Figure 1). The only other significant difference in NMR data for C13 through C23 is the chemical shift of the C13 quaternary carbinyl carbon signal at 66.4 ppm (69.95 ppm in azadirachtin). These data indicate 1 is an analogue of azadirachtin of which the C11-C13 ether linkage is reductively cleaved at C11.

(ii) The configuration of the methoxycarbonyl group attached to C11 is opposite to that of azadirachtin. This is supported by the small coupling of 9-H with 11-H which indicates the dihedral angle of H-C9-C11-H is approximately 90°. The significant low field shift of the C1 proton (5.53 ppm) compared to that of azadirachtin (4.76 ppm) is consistent with the removal of the anisotropic effect caused by the C11 methoxycarbonyl group. The methyl proton signal due to C19 shifting upfield (1.45 ppm) compared to that (1.72 ppm) of azadirachtin can also be explained by the difference of the configuration at C11.

(iii) The lack of ^{1}H and ^{13}C NMR signals corresponding to the acetoxyl group in azadirachtin and the chemical shift of 3-H (3.50 ppm vs 5.49 ppm in 2) indicate 1 contains a 3α-OH group instead of the corresponding acetate in 2.

The structure of the new limonoid was thus defined as shown in 1 and named deacetyl azadirachtinol. The ^{1}H and ^{13}C NMR assignments of deacetylazadirachtinol were greatly facilitated by the ^{1}H-^{1}H and ^{1}H-^{13}C 2D NMR spectra. The carbon sequences (C1-C3, C5-C7, C9-C11, C15-C17 and C22-C23) corresponding to the continuous proton systems were readily established by the ^{1}H-^{13}C 2D spectrum as shown in Figure 2. Especially, the unequivocal distinguish between the carbon signals (C1/C3, C18/C19, C21/C22, C30/C32, and C4'/C5') has been achieved without the aid of any other combined techniques. For example, the 3'-methyl signal at 1.80 ppm (dq, 7, 1) and the 2'-methyl signal at 1.84 ppm (dq, 1, 1.5) were observed to correlate with the carbon resonances at 14.5 and 11.9 ppm, respectively, thus providing an unequivocal assignment of the two vinyl methyl signals. In a similar manner, the assignment of the two methylene carbon signals at C-30 and C-32 was also achieved, confirming the uncertain assignment made previously[3]. The total assignment of carbon signals in deacetylazadirachtinol is listed in Table 1.

The biological activity of deacetylazadirachtinol will be reported elsewhere together with other limonoids.

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Table 1. ^{1}H and ^{13}C NMR data of deacetylazadirachtinol (1) and azadirachtin (2)[3] in $CDCl_3$.

^{1}H	1	2	^{13}C	1	2	^{13}C	1	2
1-H	5.53(t,3)	4.76(t,2.5)	C-1	67.5(d)	70.51(d)	C-1'	166.9(s)	166.1(s)
2-H	2.30(ddd,3,3,16.5)	2.30(m)	C-2	31.9(t)	29.37(t)	C-2'	128.3(s)	128.6(s)
	2.06(m)	-	C-3	69.2(d)	66.99(d)	C-3'	138.7(d)	137.5(d)
3-H	3.50	5.49(t,2.5)	C-4	43.9(s)	45.41(s)	C-4'	14.5(q)	14.29(q)
5-H	3.31(d,12.5)	3.35(d,12)	C-5	35.0(d)	37.06(d)	C-5'	11.9(q)	11.94(q)
6-H	4.57(dd,3,12.5)	4.58(dd,3,12)	C-6	76.0(d)	74.37(d)	OMe	52.5(q)	52.72(q)
7-H	4.70(d,3)	4.62(d,3)	C-7	73.5(d)	76.43(d)		52.9(q)	53.52(q)
9-H	3.18(brs)	3.34	C-8	51.1(s)	50.19(s)	OAc	-	21.33(q)
11-H	4.47 (brs)	-	C-9	43.6(d)	44.69(d)		-	169.5(s)
15-H	4.61(d,3.5)	4.70(d,2.5)	C-10	53.2(s)	52.52(s)			
16-H	1.68(ddd,3.5,5.5,13)	1.7	C-11	79.3(d)	104.1(s)			
	1.33(d,13)	1.31(d,12)	C-12	173.3(s)*	171.1(s)*			
17-H	2.34(d,5.5)	2.38(d,6.0)	C-13	66.4(s)	69.95(s)			
18-H	2.04(s)	2.06(s)	C-14	69.4(s)	68.53(s)			
19-H	1.45(s)	1.72(s)	C-15	74.2(d)	73.79(d)			
21-H	5.66(s)	5.64(s)	C-16	24.9(t)	25.06(t)			
22-H	5.05(d,3)	5.05(d,2.5)	C-17	48.8(d)	48.67(d)			
23-H	6.45(d,3)	6.42(d,2.5)	C-18	18.1(q)	20.88(q)			
30-H	3.82(d,9)	3.75	C-19	21.2(q)	18.40(q)			
	4.05(d,9)	4.05	C-20	83.5(s)	83.55(s)			
32-H	3.47(d,9.5)	3.65	C-21	109.0(d)	107.3(d)			
	3.94(d,9.5)	4.16	C-22	107.4(d)	108.7(d)			
3'-H	6.94(qq,7,1.5)	6.85(m,6)	C-23	146.6(d)	147.0(d)			
4'-H	1.80(dq, 7,1)	1.78(d,6)	C-30	73.2(t)	72.99(t)			
5'-H	1.84(dq,1.5,1)	1.84	C-31	173.9(s)*	173.2(s)*			
OAc	-	1.92(s)	C-32	71.3(t)	69.07(t)			
OMe	3.74(s)	3.76(s)						
	3.74(s)	3.65(s)						

*Assignments could be reversed.

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