PREPARATION OF 3-DEACETYLAZADIRACHTIN USING AZADIRACHTIN ISOLATED FROM *AZADIRACHTA INDICA* SEED

Azizol Abdul Kadir

forest Research Institute Malaysia, Kepong 52109 Kuala Lumpur, Malaysia

&

J.D. Connolly

Chemistry Department, University of Glasgow, G12 8QQ, United Kingdom

Received November 1990

AZIZOL ABDUL KADIR & CONNOLLY J.D. 1991. Preparation of 3-deacetyl-azadirachtin using azadirachtin isolated from *Azadirachta indica*seed. Extraction of *Azadirachta indica* or "neem" seed with organic solvents afforded azadirachtin, a strong locust antifeedant and insect growth inhibitory compound. Base hydrolysis of a/.adirachtin yielded 16.7% 3-deacetyla/,adirachtin after esterification and reacetylation gave 56% azadirachtin.

Key words: Azadirachtin - antifeedant - hydrolysis - esterification - 3-deacetyl-azadirachtin - reacetylation

Introduction

Azadirachta indica (Meliaceae) tree, known in the vernacular as "neem" or "mambu" is widely distributed in Asia, Africa and has been introduced into many other tropical parts of the world. Almost every part of the tree is used for traditional medicine particularly in India. Azadirachtin (1) (Figure 1) is one of the most interesting and complex compounds isolated from this species because of its strong locust antifeedant and insect growth inhibitory properties. These properties have been reported by workers such as Jacobson (1986) and many others in the Proceedings of the First (1980), Second (1983) and Third (1986) International Neem Conferences, edited by Schmutterer *et al.* (1981), Schmutterer & Ascher (1984, 1987) respectively. Since the correct structure of azadirachtin (1) was recently established by high fields Nuclear Magnetic Resonance (NMR) (Kraus *et al.* 1985, Turner *et al.* 1987) and X-ray crystallography (Broughton *et al.* 1986), there is growing interest on the chemical characterization of the funtional groups and synthesis of azadirachtin (1). Furthermore, chemical modification of azadirachtin (1) and its derivatives has been carried out to study their biological activity (Ley *et al.* 1987, 1988). However, azadirachtin (1) is unstable and will degrade, especially if kept too

long in solution (Azizol & Connolly 1990, Zanno *et al.* 1975). Degradation studies of azadirachtin (1) by UV light (Ermel *et al.* 1986) showed that azadirachtin (1) content was reduced by 65% within 14 *h* exposure to UV light. Thus, a stable azadirachtin derivative is necessary for detailed investigation of the mode of specific functional groups and also for bioassay.

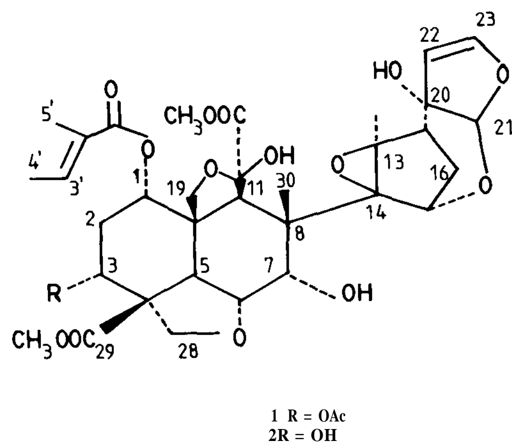

Figure 1. Structures of azadirachtin (1) and 3-deacetylazadirachtin (2)

Materials and methods

Dried seeds of *A. indica* were obtained from Pakistan and ground with a Wiley Mill. The extraction, isolation and purification methods are described elsewhere (Azizol & Connolly 1990).

Preparation of 3-deacetylazadirachtin (2)

Various concentrations of potassium hydroxide (KOH) were tried for the hydrolysis of azadirachtin (1). It was believed that 2.0% KOH, which was suggested by Zanno *et al.* (1975), was too strong for the hydrolysis since no products were detected. The following two methods of hydrolysis were successful:

Hydrolysis of azadirachtin (1) with 1% **KOH.** Azadirachtin (1) (128.5 *mg)* in methanol (7.0 *ml)* was treated with aqueous 1% KOH solution (4.0 *ml)* for 3.5 *h* at room temperature. The mixture was made just acidic with dilute hydrochloric acid and extracted with ethyl acetate (4 x 10.0 *ml).* Evaporation of the solvent gave a crude acidic product (88.5 *mg).*

Hydrolysis of azadirachtin (1) with sodium carbonate. Azadirachtin (1) (68.2 *mg)* in methanol (3.0 *ml)* was added to sodium carbonate (23.5 *mg)* in water (3.0 *ml)* and was allowed to stand for three days. The reaction mixture was made

just acidic with dilute hydrochloric acid and extracted with ethyl acetate (4 x 10.0 *ml)*. Evaporation of the solvent gave a crude acidic product (55.1 *mg)*.

Esterification of the acidic products

Chloroform solutions of both crude acidic products were esterified by addition of excess diazomethane. The esterified product was then purified by preparative thin layer chromatography (t.l.c.) [petroleum ether (60-80°C) : ethyl acetate, 1 : 4, three runs] and afforded 16.7 and 8.8% of 3-deacetylazadirachtin (2) by methods 1 and 2 respectively. The 3-deacetyl-azadirachtin (2) was crystallised from CCl_4 as a microcrystalline powder. 3-Deacetylazadirachtin (2) was kept for two months at room temperature (18°C) and the purity was checked by analytical t.l.c. which showed only one spot.

Reacetylation of the 3-deacetylazadirachtin (2)

The 3-deacetylazadirachtin (2) (8.9 *mg)* was acetylated with acetic anhydride (0.5 *ml)* and pyridine (0.4 *ml)*. The reaction mixture was allowed to stand overnight at room temperature. After removing the excess acetic anhydride and pyridine, the crude product was purified by t.l.c. [petroleum ether (60-80°C) : ethyl acetate, 2 : 3] and afforded azadirachtin (1)(5.0 *mg),* identical to the authentic material by 'H NMR and analytical t.l.c. (R_r 0.5 in ethyl acetate).

Instrumentation

All melting points (m.p.) which were uncorrected, were determined on a Kofler hot-stage apparatus. Infrared (i.r.) spectra were recorded in CCl_4 solution on Perkin Elmer 580. Kieselgel GF<„4 was used for preparative and analytical t.l.c. (0.5 *mm* thickness). Analytical t.l.c. plates were visualised by heating for several minutes after spraying with eerie sulphate. NMR spectra were recorded for CDC1, using Bruker WP200SYinstrument ('H 200 *MHz,* ^{1S}C 50.32 MHz). Azadirachtin (1): m.p. 150"*C,* $[oc]_D$ -38" (c, 3.44 in CHC1,), [Lit m.p. 156"C, $[a]_D$-46"], i.r. u_{max} cm^{1} 3450(br), 1730, 1720, 1648, 1620. 'H NMR (see Table 1). 3-DeacetylazadTrachtin (2); m.p. 143-145"C, $[a]_D$ -32" (c, 0.49 in $CHCl_3$), i.r. $i)_{max}$ $cm^{'1}$ 3480, 1645. 'H NMR (see Table 1), ^{13}C NMR (see Table 2).

Results and discussion

It has been reported earlier that 3-deacetylazadirachtin (2) could be prepared using 2.0% KOH (Zanno *et al.* 1975) and sodium methoxide (Rembold *et al.* 1986). Several attempts at the preparation of 3-deacetylazadirachtin (2) and 2% KOH failed to yield any hydrolysed product after esterification with diazomethane. The reaction clearly needs higher amounts of azadirachtin (1). However, hydrolysis using 1 % KOH for 3.5 *h* afforded 16.7% of 3-deace-

tylazadirachtin (2) after esterification of the crude acidic product. This yield was much higher than that obtained with 2-equivalents of sodium carbonate (8.8%) and sodium methoxide (15.2%) (Rembold *et al.* 1986). The structure of 3-deacetylazadirachtin (2) was established by comparison of its ^{1}HNMR spectrum with that of azadirachtin (1) (Table 1). The most obvious differences are the lack of an acetate signal and the upfield shift of H-3 (δ_H 4.36). In the ^{13}C NMR spectrum of 3-deacetylazadirachtin (Table 2), C-3 (δ_C 71.6) moves downfield compared to C-3 of azadirachtin (δ_C 66.99) (Kraus *et al.* 1985). Detailed comparison with the ^{1}H and ^{13}C NMR spectra of azadirachtin (1) showed that most other signals remained unchanged. One interesting feature about 3-deacetylazadirachtin (2) was its stability at normal conditions where it was not degraded when kept for more than two months.

Table 1. ^{1}H NMR chemical shifts of azadirachtin (1) and 3-deacetylazadirachtin (2)

H-Atom	Azadirachtin (1)	3-deactylazadirachtin (2)
1-H	4.75 (dd, J=2.8, 3.0 *Hz)*	4.84 (t,2.7 *Hz)*
2-H	2.27 (add, J=16.5,3.0,2.7 *Hz)*	2.25 (dt,J=16.5,2.7 *Hz)*
2-H	2.10 (ddd,J=16.5, 2.9, 2.9 *Hz)*	2.67 (m)
3-H	5.47 (dd,J=2.5, 2.7 *Hz)*	4.36 (dt,J=7.3, 3.1 *Hz)*
5-H	3.38 (J=12.5 *Hz)*	3.21 (dd,J=12.5, 2.8 *Hz)*
6-H	4.60 (dd,J=12.5, 2.6 *Hz)*	n.d.
7-H	4.75 (d, 2.6 *Hz)*	4.62 (dd, J=12.5, 2.8 *Hz)*
9-H	3.30 (s)	4.65 (d,J=3.5 *Hz)*
15-H	4.65 (d,J=3.0 *Hz)*	4.65 (d,J=3.5 *Hz)*
16-H	1.70 (ddd,J= 13.5, 5.0, 4.0 *Hz)*	1.66 (ddd,J=13.3, 5.4, 3.9 *Hz)*
16-H	1.22 (d, 1=13.5 *Hz)*	1.29 (d,J=5.2 *Hz)*
17-H	2.35 (d,J=5.0 *Hz)*	2.38 (d,J=5.2 *Hz)*
18-H	1.98 (s)	2.01 (s)
19-H	3.62 (d, J=9.5 *Hz)*	3.58 (d,J=9.8 *Hz)*
19-H	4.16 (d,J=9.5 *Hz)*	4.22 (d, J=9.8 *Hz)*
21-H	5.65 (s)	5.65 (s)
22-H	5.05 (d, J=3.0 *Hz)*	5.04 (d,J=2.9 *Hz)*
23-H	6.43 (d,J=3.0 *Hz)*	6.44 (d,J=2.9 *Hz)*
28-H	4.05 (d,J=9.0 *Hz)*	4.06 (d,J=8.6 *Hz)*
28-H	3.74 (d,J=9.0 *Hz)*	3.61 (d, J=8.6 *Hz)*
30-Me	1.72 (s)	1.75 (s)'
7-OH	3.05 (s)	2.75 (s, br)
11-OH	5.07 (s)	5.02 (s)
20-OH	3.20 (s)	2.93 (s, br)
12-OMe	3.65 (s)	3.76 (s)
29-OMe	3.76 (s)	3.88 (s)
OAc	1.95 (s)	-
3'-H	6.93 (qq,J=7.5, 1.5 *Hz)*	6.83 (qq,J=7.1, 1.4 *Hz)*
4'-H	1.75 (dq,J=7.5, 1.5 *Hz)*	1.78 (dq,J=7.1, 1.1 *Hz)*
5'-H	1.81 (dq,J=1.5, 1.1 *Hz)*	1.84 (dq,J=1.2, 1.3 *Hz)*

n.d. - not detected

Reacetylation of 3-deacetylazadirachtin (2) with acetic anhydride and pyridine proceeded smoothly to afford azadirachtin (1)(56.0%), identified by ^{1}H NMR and analytical t.l.c. and comparison with standard azadirachtin (1).

Table 2. "C NMR chemical shifts of 3-deacetylazadirachtin (2)

C-Atom	
C-1	66.0 (s)
C-2	32.0 (t)
C-3	71.6 (d)
C-4	53.3 (s)
C-5	35.3 (d)
C-6	73.7 (d)
C-7	75.8 (d)
C-8	52.4 (s)
C-9	44.7 (d)
C-10	52.5 (s)
C-11	104.2 (s)
C-13	69.8 (s)
C-14	69.4 (s)
C-15	77.0 (d)
C-16	25.1 (t)
C-17	48.7 (d)
C-18	18.3 (q)
C-19	71.8 (t)
C-20	83.5 (s)
C-21	108.7 (d)
C-22	107.4 (d)
C-23	147.0 (d)
C-28	73.0 (t)
C-30	21.2 (q)
12-OMe	53.2 (q)
29-OMe	52.5 (q)
C-2'	128.1 (s)
C-3'	138.3 (d)
C-4'Me	14.5 (q)
C-5'Me	12.1 (q)

C-12 and C-1' not detected (n.d.)

Conclusion

The successful preparation of 3-deacetylazadirachtin (2) and reacetylation opens the way for the preparation of labelled azadirachtin using C^{14}-acetic anhydride and pyridine. The formation of labelled azadirachtin makes possible detailed investigations related to the bioassay and characteristics of functional groups of azadirachtin (1) in the future.

Acknowledgements

This paper is part of the Ph. D thesis of the senior author who would like to thank FRIM for granting his study leave. We wish to thank E. Strang, Department of Biochemistry, University of Glasgow for supplying the neem seeds.

References

AZIZOL ABDUL KADIR & CONNOLLY, J.D. 1990. Isolation and identification of limonoids from *Azadirachta indica* seeds. Pp. 93-102 in Sam, T.W. (Ed.) *Proceedings of the Seventh National Seminar on Natural Products.* June 27 - 28, 1990. Universiti Sains Malaysia.

BROUGHTON, H.B., LEY, S.V., SLAWIN, A.M.Z., WILLIAMS, DJ. & MORGAN, E.D. 1986. X-ray crystallographic structure determination of ditigloydihydroazadirachtin and reassignment of the limonoid insect antifeedant *azadirachtin. Journal of Chemical Society Chemical Communications* 46-47.

ERMEL, K., PAHLICH, E. & SCHMUTTERER, H. 1986. Azadirachtin content of neem kernels from different geographical locations, and its dependence on temperature, relative humidity, and light. Pp 171-184 in Schmutterer, H. & Ascher, K.R.S. (Eds.) *Proceedings of the Third International Neem Conference.* July 10 - 15, 1986. Nairobi, Kenya.

JACOBSON, M. 1986. Natural resistance of plants to pests. Pp. 220 in Green, M.B. & Hedin, P.A. (Eds.) *ACS Symposium 296.*

KRAUS, W., BOKEL, M., KLENK, A. & POHNL, H. 1985. The structure of azadirachtin and 22,23-dihydro-23 -methoxy-azadirachtin. *Tetrahedron Letters 26* (52): 6435-6438.

LEY, S.V., SANTAFIANOS, D., BLANEY, W.M. & SIMMONDS, M.S.J. 1987. Synthesis of a hydroxy dihydrofuran acetal related to azadirachtin. *Tetrahedron Letters* 28 (2): 221-224.

LEY, S.V., ANDERSON, J.C., BLANEY, W.M., LIDERT, Z., MORGAN, E.D., ROBINSON, N.G. & SIMMONDS, S.J. 1988. Chemistry of insect antifeedants from *Azadirachta indica* (Part 3): Reaction on the C-22,23 enol ether double bond of azadirachtin and conversion to 22,23-dihydro-23 -methoxyazadirachtin. *Tetrahedron Letters 29* (42): 5433-5436.

REMBOLD, H., FORSTER, H. & ZOPPELT, C. 1986. Structure and biological activity of azadirachtins A and B. Pp. 149 - 160 in Scmutterer, H. & Ascher, K.R.S. (Eds.) *Proceedings of the Third International Neem Conference.* July 10 - 15, 1986. Nairobi, Kenya.

SCHMUTTERER, H., ASCHER, K.R.S. & REMBOLD, H. (Eds.) 1981. Natural pesticide from the neem tree. *Proceedings of the First International Neem Conference. June* 16 - 18,1980. Rottach-Egern, Federal Republic of Germany.

SCHMUTTERER, H. & ASCHER, K.R.S. (Eds.) 1984. Natural pesticides from the neem tree and other tropical plants. *Proceedings of the Second International Neem Conference.* May 25 - 28, 1983. Rauischholghausen, Federal Republic of Germany.

SCHMUTTERER, H. & ASCHER, K.R.S. (Eds.) 1987. Natural pesticides from the neem tree and other tropical plants. *Proceedings of the Third International Neem Conference. July* 10 - 15, 1986. Nairobi, Kenya.

TURNER,CJ., TEMPESTA, M.S., TAYLOR, R.B., ZAGORSKI, M.G., TERMINI.J.S., SCHROEDER, D.R. & NAKANISHI, K. 1987. An NMR spectroscopic study of azadirachtin and its trimethyl ether. *Tetrahedron Letters* 43 (12): 2789-2803.

ZANNO, P.R., MINRA , I. & NAKANISHI, K. 1975. Structure of the insect phagorepellent azadirachtin. Application of PRFT/CWD Carbon-13 Nuclear Magnetic Resonance. *Journal of American Chemical Society* 97: 1975-1976.