Pergamon

Tetrahedron Letters 40 (1999) 5243–5246

TETRAHEDRON LETTERS

Biocatalyst-mediated expansion of ring D in azadirachtin, a potent insect antifeedant from *Azadirachta indica*

K. M. Madyastha*[1,2] and K. Venkatakrishnan[1]

[1]*Department of Organic Chemistry, Indian Institute of Science, Bangalore-560012*

[2]Chemical Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore-560064.

Received 30 March 1999; accepted 13 May 1999

Abstract

A microorganism identified as *Nocardia Sp.* capable of converting azadirachtin **1** into three metabolites, viz. 3-deacetylazadirachtin **2**, 1-detigloyl-3-deacetyl-azadirachtin-1-ene-3-one **3** and 1-detigloyl-3-deacetyl-11,19-deoxa-12,19-oxa-11-oxo-azadirachtin-1-ene-3-one **4**, has been isolated. This is the first report on the functionalization of azadirachtin using biocatalysts wherein two novel, hitherto unknown metabolites have been isolated and characterized.

Keywords; *Azadirachta indica*; Azadirachtin; biocatalyst; *Nocardia Sp.*; α,β-unsaturated ketone; α-keto-δ-lactone.

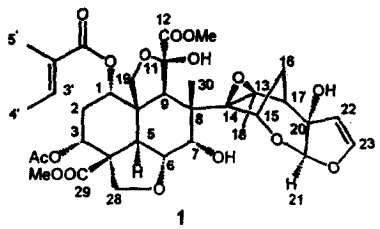
Azadirachtin, the tetranortriterpene from *Azadirachta indica A. Juss* (neem tree) has generated wide academic and industrial interest because of its powerful antifeedant and hormonal activity towards many species of insects. In fact a considerable amount of work has already been carried out on structure–activity relationships [1-4]. Although there are several reports on skeletal and functional group conversions of azadirachtin and related compounds using synthetic reagents [5,6], transformations mediated by biocatalysts have not been reported so far.

Our quest for developing biologically active and stable derivatives of azadirachtin and related compounds using biocatalysts, led to the isolation of a gram positive bacterium identified as *Nocardia Sp.* which utilizes azadirachtin as the sole source of carbon. This organism has been shown to bring about efficient transformation in salannin, a tetranortriterpene [7]. In the present studies we have shown that the organism is capable of converting azadirachtin **1** into 3-deacetylazadirachtin **2**, 1-detigloyl-3-deacetyl-azadirachtin-1-ene-3-one **3** and 1-detigloyl-3-deacetyl-11,19-deoxa-12,19-oxa-11-oxo-azadirachtin-1-ene-3-one **4**. The metabolites **3** and **4** are found to be more stable than the parent compound **1**. These novel hitherto unknown metabolites (**3**,**4**) having an α,β-unsaturated ketone moiety in ring A with or without a lactone functionality between C-12 and C-19, could prove to be better antifeedants and cytotoxic agents than azadirachtin **1**. In fact earlier it was demonstrated that

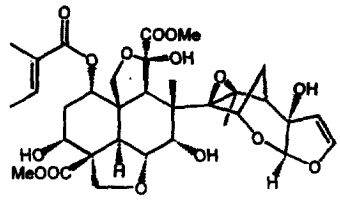
0040-4039/99/$ - see front matter
PII: S0040-4039(99)00992-2

gedunin, one of the minor constituents of neem, with an α,β-unsaturated ketone as part of the ring A and a δ-lactone moiety in its structure, has significant antifungal and antimalarial activities [8].

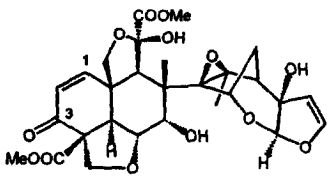
Nocardia cells were grown for 36 h in a mineral salts medium (pH 7.2) containing glucose (0.2%) and yeast extract (0.05%) following standard procedures [7]. Azadirachtin **1** (500 mg, 0.7 mmol) was incubated with the resting cells of *Nocardia sp.*† (~ 4g wet weight in 125 ml of phosphate buffer, 0.01M, pH 7.2) on a rotary shaker at 29-30°C for 2-8 h. At the end of the incubation period, the cell suspension was centrifuged, the supernatent acidified to pH 6-6.5 and extracted with ethyl acetate. The ethyl acetate layer was dried and subjected to column chromatography (silica gel) using hexane-EtOAc as the solvent system to separate the metabolites. The ratio of metabolites formed depends upon the duration of incubation. During the early stages of incubation (2h), the major metabolite (60%) formed was characterized as 3-deacetylazadirachtin **2**. The ^{1}H NMR of this metabolite clearly showed the absence of the peak at δ 1.95 indicating that deacetylation of **1** had occurred. This was further confirmed from the mass spectrum of **2** [9].



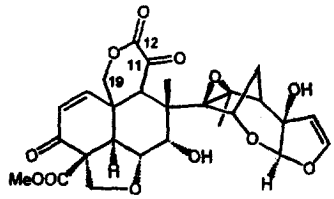
1



2



3



4

Prolonging the period of incubation (6h) results in the formation of metabolite **3**. The IR spectrum of this metabolite showed peaks at 1735 cm^{-1} and 1692 cm^{-1}. The ^{1}H NMR spectrum of this compound was similar to that of azadirachtin **1** in the following spin systems,

i) the three spin system of H-5, H-6, and H-7;

ii) the three spin system of H-15, 16α,β and H-17;

iii) H-22, H-23 of dihydrofuran ring,

iv) the AB systems H-19α,β and H-28α,β

†Details of the incubation conditions and separation of the metabolites will be described in a full paper

v) Methyls at H-18 and H-30;
vi) the two carbomethoxyl groups at C-4 and C-1

Unlike the spectrum of azadirachtin, the metabolite exhibited additional signals at δ 5.97 and δ 6.90. Also it was seen from the ^{1}H-^{1}H COSY spectrum that these two signals were coupled to each other. The slight variation in the chemical shifts of the AB systems H-19 and H-28 combined with the missing signals for tigloyl as well as the acetate strongly suggested that the changes had taken place in the 'A' ring of the azadirachtin skeleton. This was also evident from the signals at δ 153 and δ 128.3 in the ^{13}C NMR, corresponding to the β and α carbons (olefinic) of an α,β-unsaturated ketone. The signal at δ 192.7 was assigned to the carbonyl at C-3. The multiplicities were established by SEFT experiments. The M^{+} was found to be 576 from FAB and electrospray mass spectral data. As rest of the signals were similar to that of **1**, the α,β-unsaturated ketone can only be accommodated in the 'A' ring of azadirachtin, the metabolite **3** was assigned as 1-detigloyl-3-deacetyl-azadirachtin-1-ene-3-one [10].

When the period of incubation was further increased (8h) the levels of metabolites **2** and **3** were significantly decreased with the appearance of a new metabolite **4**.

The IR spectrum of metabolite **4**, showed the peaks at 1741 cm^{-1} and 1693 cm^{-1}. The ^{1}H NMR of this metabolite differs from azadirachtin **1** in the following signals;
i) signals corresponding to the tigloyl moiety at C-1, the acetate group at C-3 and the methylene at C-2 were missing;
ii) the presence of two doublets which appear far downfield and are coupled to each other (^{1}H-^{1}H COSY), suggests the presence of an α,β-unsaturated ketone;
iii) the absence of one of the two carbomethoxyl groups (the methyl signal in the COOMe) suggests the possible rearrangement at either C-4 or C-11
The absence of the signal corresponding to C-11 in the ^{13}C NMR of **4**, clearly indicated that the changes have taken place around this carbon. The signals at δ 189.40 and δ 192.53 in the ^{13}C NMR, indicated the presence of two carbonyl functionalities in addition to the one present in the ester group. Furthermore it was also observed from the ^{1}H NMR spectrum that some of the signals had a considerable change in their chemical shifts (for eg. H-6 and H-19). This clearly points out that the rearrangement has taken place around C-11 in the five membered lactol system. Thus the newly formed carbonyl functions of the α-keto-δ- lactone system can exert an anisotropic effect over the nearby protons, thereby explaining the abnormal upfield shifts for H-6 [δ 4.08-4.14 (m); δ 4.60 in **1**], H-30 [δ 1.68 (s); 1.74 in **1**] and the downfield shift for H-19 [δ 5.31 (d), H-19b; δ 4.15 (d) in **1**] observed in the ^{1}H NMR spectrum. FABMS studies showed the M^{+} to be at 567 and the C,H,N analysis of the compound indicated the formula to be $C_{27}H_{28}O_{12}$ Based on the spectral analyses, metabolite **4** was identified as 1-detigloyl-3-deacetyl-11,19-deoxa-12,19-oxa-11-oxo-azadirachtin-1-ene-3-one [11].

Acknowledgements

The SIF at IISc for recording the NMR spectra. Financial assistance from CSIR (New Delhi) and JNCASR

(Bangalore) is gratefully acknowledged. Financial support to K.V. by the UGC is greatly appreciated.

References and notes

1. Kraus W : Schmutterer H, editor. *The Neem Tree, Azadirachta indica A. juss and other Meliaceous Plants,* Weinheim, Verlag Chemie, 1995: 35-88.
2. Bilton JN, Jones PS, Ley SV, Robinson NG and Sheppard RN. Tetrahedron Lett. 1988;29:1849-1852.
3. Ley SV, Anderson JC, Blaney WM, Zev Lidert, Morgan ED, Robinson NG, Simmonds MSJ.Tetrahedron Lett., 1988; 29:5433-5436.
4. Ley SV, Anderson JC, Blaney WM, Jones PS, Zev Lidert, Morgan ED, Robinson NG, Dinos Santafianos, Simmonds MSJ and Toogood PL. Tetrahedron, 1989:5175-5192.
5. Ley SV, Anderson JC, Blaney WM, Morgan ED, Sheppard RN, Simmonds MSJ, Slawin AMZ, Smith SC, Williams DJ and Wood A, Tetrahedron, 1991; 47:9231-9246.
6. Ley SV, Lovell PJ, Slawin AMZ, Smith SC, Williams DJ and Wood A. Tetrahedron, 1993;49:1675.
7. Venkatakrishnan K and Madyastha KM. J. Chem. Soc., Perkin Trans 1, 1998:1183-1184.
8. Khalid SA, Duddeck H and Gonzalez-Sierra M. *J. Nat. Prod.*, 1989, 52, 922-927.
9. Yamasaki RB, Klocke JA. J. Agric. Food Chem. 1987, 35, 467-471.
10. Selected data for 3: ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.33 (1H, d, J=13Hz, H-16b), 2.02 (3H, s, H-18), 3.19 (1H, d, J=12Hz, H-5), 3.74 (3H, s, COOMe), 3.87 (3H, s, COOMe), 3.78 (1H, d, J=9Hz, H-28α), 4.16 (1H, d, J=9Hz, H-19α), 4.26 (1H, d, J=9Hz, H-28β), 4.40 (1H, d, J=9Hz, H-19β), 4.54 (1H, dd, J=12, 3Hz, H-6), 4.66 (1H, bs, H-7), 5.04 (1H, d, J=3Hz, H-22), 5.63 (1H, s, H-21), 5.98 (1H, d, J=10Hz, H-2), 6.45 (1H, d, J=3Hz, H-23), 6.90 (1H, d, J=10Hz, H-1). ^{13}C NMR ($CDCl_3$,125 MHz): δ 18.1 (C-18), 21.0 (C-30), 25.1 (C-16), 45.7 (C-8), 46.5 (C-5), 48.4 (C-9), 53.1 (C-29, C-11 OMe), 58.4 (C-4), 69.1 (C-14), 69.3 (C-13), 73.4 (C-6), 74.4 (C-7), 76.0 (C-15), 83.3 (C-20), 104.3 (C-11), 107.4 (C-22), 108.4 (C-21), 128.3 (C-2), 146.8 (C-23), 153.5 (C-1), 171.3 (C-29), 171.9 (C-12), 192.7 (C-3). MS-ES: 576.7 (M^+), 559.5, 541.5, 354.5.
11 Selected data for 4: ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.29 (1H, d, J=13.0 Hz, H-16b), 1.58 (1H, brs, H-16a), 1.68 (3H,s, H-30), 1.97 (3H, s, H-18), 2.36 (1H, d, J=5.2 Hz, H-17), 3.92 (3H, s, 29-Ome), 4.08-4.14 (1H, m, H-19a), 4.08-4.14 (1H, m, H-6), 4.33 (1H, d, J=9.8 Hz, H-28a), 4.48 (1H, d, J=3.4Hz, H-15), 4.61 (1H, d, J=9.8 Hz, H-28b), 4.68 (1H, d, J=2.3 Hz, H-7), 5.04 (1H, d, J=2.8 Hz, H-22), 5.31 (1H, d, J=13.2 Hz, H-19b), 5.63 (1H, s, H-21), 6.17 (1H, d, J=9.8 Hz, H-2), 6.43 (1H, d, J=2.8 Hz, H-23), 7.08 (1H, d, J=9.8 Hz, H-1): ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.93 (C-18), 23.52 (C-30), 25.77 (C-16), 30.14 (C-4), 38.78 (C-8), 46.65 (C-5), 47.00 (C-9), 49.12 (C-17), 52.07 (C-10), 54.19 (C-29 OMe), 59.74 (C-28), 69.53 (C-13), 70.23 (C-14), 72.13 (C-19), 74.37 (C-6), 75.61 (C-7), 76.45 (C-15), 84.31 (C-20), 108.40 (C-22), 109.16 (C-21), 130.60 (C-2), 147.83 (C-23), 151.37 (C-1), 163.84 (C-12), 165.56 (C-29), 189.41 (C-3), 192.53 (C-11)
FABMS: 567 (M^+), 527, 443. (Found C, 55.8824; H, 5.8085; O, 38.3091: $C_{27}H_{28}O_{12}.2H_2O$ requires C, 55.8621; H, 5.5172; O, 38.6207).