COMMUNICATION

Biocatalyst-mediated efficient functionalization of ring A in salannin, a tetranortriterpene from *Azadirachta indica*

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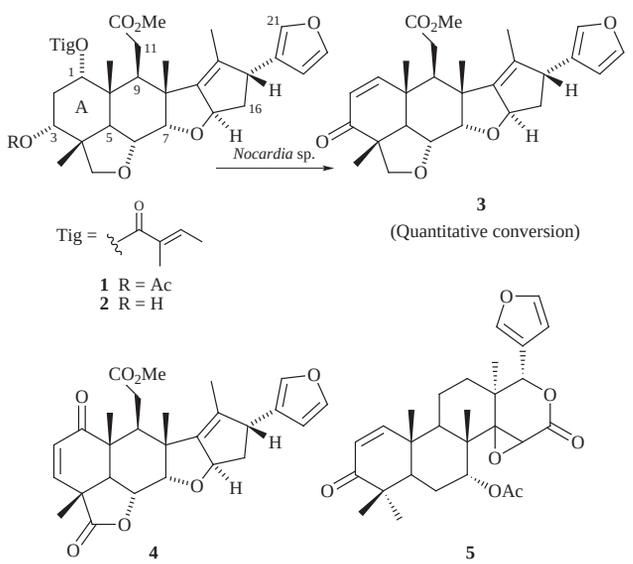
Salannin 1 and deacetylsalannin 2 have been quantitatively converted into 1-detigloyloxy-3-deacetylsalannin-1-en-3-one 3,† a hitherto unknown compound, in a single step using *Nocardia* sp. and the pathway for the transformation has been investigated.

Limonoids from *Azadirachta indica* A. Juss (neem tree) are of great interest as they exhibit a variety of biological properties.[1–4] One of the potent cytotoxic compounds in neem is nimbolide **4** and it has been shown that the presence of an α,β-unsaturated ketone in ring A is largely responsible for the observed biological activity.[5,6] This compound and another minor neem constituent, gedunin **5**, have been shown to have antimalarial

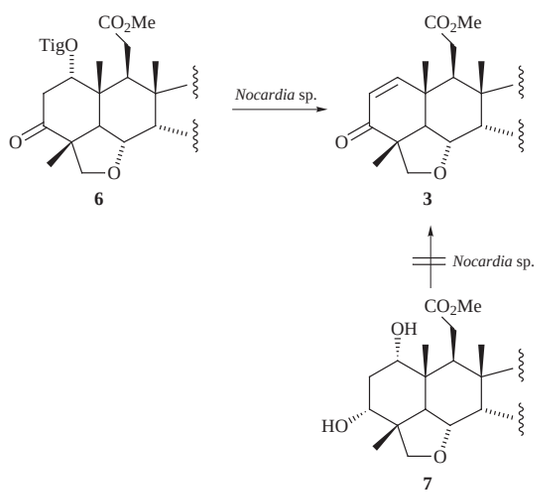


activity[7,8] and it is interesting to note that both these compounds have a certain degree of chemical resemblance, particularly in the ring A pattern.[9,10] However, salannin **1** is one of the major limonoids present in the neem seed which does not possess significant biological activity.[6] In our effects to prepare limonoids having an α,β-unsaturated ketone moiety in ring A without affecting the rest of the molecule, we have isolated a Gram positive bacterium identified as *Nocardia* sp. capable of converting salannin **1** and 3-deacetylsalannin **2** quantitatively into 1-detigloyloxy-3-deacetylsalannin-1-en-3-one **3**, a compound hitherto not known. Such a transformation would be difficult to carry out using a conventional approach. The present paper describes the sequence of events leading to the formation of **3** and its complete characterization.

† Tigloyl = C(O)C(Me)=CHMe.

Results and discussion

Salannin **1** and 3-deacetylsalannin **2** when incubated with *Nocardia* sp. yielded compound **3**, which was characterized as 1-detigloyloxy-3-deacetylsalannin-1-en-3-one. Further investigation revealed that the first step in the transformation of salannin to the product **3**, was deacetylation. This was evident from the ready conversion of 3-deacetylsalannin into **3**. The next step in the transformation seems to be the oxidation of the secondary hydroxy group of 3-deacetylsalannin **2** to 3-oxo-3-deacetylsalannin **6** (Scheme 1). In fact this compound when incubated



Scheme 1

in *Nocardia* sp. yielded **3**, suggesting its intermediacy in the formation of **3**. 1-Detigloyloxy-3-deacetylsalannin **7**, when incubated with the organism does not get converted into **3** ruling out its possible intermediacy in the pathway. Preliminary studies indicated that compound **3** showed antimalarial activity when tested *in vitro* against strains of *Plasmodium falciparum* (unpublished observation).

That the metabolite formed was indeed 1-detigloyloxy-3-deacetylsalannin-1-en-3-one **3** was established based on the following spectral evidence. The IR spectrum of the compound **3**, showed a strong absorbance at 1680 cm^{-1} indicating the presence of an α,β-unsaturated ketone. The ^1H NMR spectrum of the product **3** was similar to that of **1** except that it showed the absence of tigloyl and acetyl groups. Also the presence of two additional peaks at δ 6.69 and 5.78, appearing as two doublets and exhibiting a coupling (J 10),‡ strongly suggested the presence of an α,β-unsaturated ketone moiety. The presence of the

‡ J Values are given in Hz.

α,β-unsaturated ketone was further confirmed from the ^{13}C NMR spectrum wherein the signals for the carbonyl carbon (δ 200.95) and the newly formed olefinic carbons (δ 127.46 and 154.62) were observed. The proton connectivities as established from ^{1}H–^{1}H COSY spectra and multiplicities from SEFT experiments indicated that the changes must have taken place in ring A of the salannin skeleton. The structure assigned was further supported from the mass spectrum of **3** which showed the molecular ion peak at *m*/*z* 452. The position of the ketone and the double bond in ring A was established as follows. (i) Incubation of 3-oxo-3-deacetylsalannin **6** with *Nocardia* sp. yielded compound **3** suggesting that the olefinic linkage should be between C-1 and C-2; (ii) the NMR spectra of **3** did not match with that of the naturally occurring compound 28-deoxonimbolide,[5] which is very similar to that of **3** but has a 2-en-1-one system in ring A; and (iii) although **3** had the same M^+ (452) as that of 28-deoxonimbolide, the fragmentation pattern was different.

Our results suggest that the conversion of **1** to **3** is initiated by deacetylation, followed by oxidation of the secondary hydroxy group to a ketone, prior to the removal of the tiglate group.

Experimental

Isolation of salannin and 3-deacetylsalannin

Salannin **1** and deacetylsalannin **2** were isolated from the methanolic extract of neem seed kernels of *Azadirachta indica*, following Nakanishi's procedure.[11] Fractions containing salannin and deacetylsalannin were subjected to column chromatography over silica gel using hexane–EtOAc (50 : 50, v/v) as the solvent system. Further purification was carried out by recrystallisation from hexane–EtOAc (40 : 60, v/v).

Preparation of 3-oxo-3-deacetylsalannin 6

To a solution of PCC (48.5 mg, 0.225 mmol) in dry CH_2Cl_2, 3-deacetylsalannin **2** (100 mg, 0.18 mmol) in CH_2Cl_2 was added dropwise and the reaction allowed to stir overnight. The reaction mixture was loaded onto a small pad of silica gel and eluted with $CHCl_3$–CH_3CN (85 : 15, v/v) in increasing polarity to obtain pure **6** (90 mg, 90% yield).

Preparation of 1-detigloyloxy-3-deacetylsalannin 7

To a suspension of K_2CO_3 (47 mg, 0.34 mmol) in MeOH, salannin **1** (100 mg, 0.168 mmol) was added portionwise and the resulting solution allowed to stir for 2 days at room temperature. Purification by column chromatography over silica gel using $CHCl_3$–CH_3CN (80 : 20, v/v) yielded pure **7** (30 mg, 38% yield).

General procedure for biotransformation

Nocardia sp. was grown in modified mineral salts medium[12] (pH 7.2), containing 0.05% of yeast extract supplemented with 0.25% of glucose (10% solution) to enhance the growth. After 24 h of growth ($A_{660} \approx 1.4$), salannin **1** [1 mmol dm^{-3} (60 mg)]/deacetylsalannin **2** [0.36 mmol dm^{-3} (20 mg)]/3-oxo-3-deacetylsalannin **6** [0.36 mmol dm^{-3} (20 mg)]/1-detigloyloxy-3-deacetylsalannin **7** [0.42 mmol dm^{-3} (20 mg)] in 0.4 cm^3 of MeOH was added and incubated at 28–30 °C with orbital shaking (220 rpm). The incubation mixture, monitored by TLC (for the disappearance of **1** and maximum formation of **3**), was stopped after a period of 12–15 h. The broth was extracted with ethyl acetate (2 × 75 cm^3), the organic phase dried over sodium sulfate and the solvent removed under reduced pressure. The residue was passed through a small pad of silica gel, eluting with $CHCl_3$–CH_3CN, to obtain pure **3**. Isolated yields of the product **3** obtained from **1**, **2** and **6** were 44 mg (97%), 15.8 mg (97%) and 16 mg (>97%), respectively.

Compound 3. Mp 66–69 °C; $[\alpha]_D^{23}$ +255 (*c* 1 in $CHCl_3$); λ_{max}(MeOH)/nm 248 (log ε 3.019); ν_{max}/cm^{-1} 1730 and 1680; δ_H(400 MHz; $CDCl_3$) 6.69 (1H, d, *J* 10,‡ 1-H), 5.78 (1H, d, *J* 10, 2-H), 2.66 (1H, d, *J* 12.5, 5-H), 4.07 (1H, dd, *J* 3.4, 12.5, 6-H), 4.20 (1H, d, *J* 3.4, 7-H), 2.54 (1H, dd, *J* 6.5, 15.6, 11a-H), 2.40 (1H, dd, *J* 5.6, 15.6, 11b-H), 5.34 (1H, m, 15-H), 3.58–3.60 (1H, m, 17-H), 1.64 (1H, d, *J* 1.5, 18-H), 1.23 (3H, s, 19-H), 7.14 (1H, s, 21-H), 6.14 (1H, dd, *J* 1.6, 0.75, 22-H), 7.25 (1H, t, *J* 1.6, 23-H), 3.82 (1H, d, *J* 8.2, 28a-H), 3.95 (1H, d, *J* 8.2, 28b-H), 1.13 (3H, s, 29-H), 1.28 (1H, s, 30-H), 3.47 (3H, s, CO_2Me); δ_C(100 MHz; $CDCl_3$) 154.62 (C-1), 127.46 (C-2), 200.95 (C=O), 48.91 (C-4), 49.51 (C-5), 72.48 (C-6), 86.33 (C-7), 49.78 (C-8), 45.04 (C-9), 39.97 (C-10), 31.29 (C-11), 173.47 (C=O, CO_2Me), 135.44 (C-13), 145.93 (C-14), 87.93 (C-15), 41.48 (C-16), 49.54 (C-17), 12.95 (C-18), 17.58 (C-19), 126.87 (C-20), 138.78 (C-21), 110.32 (C-22), 143.10 (C-23), 77.09 (C-28), 18.32 (C-29), 15.45 (C-30), 52.00 (CO_2Me); *m*/*z* (EI) 452 (M^+) (HRMS: found M^+, 452.2200. Calc. for $C_{27}H_{32}O_6$, 452.2199).

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