PERKIN 1

Structural flexibility in the biocatalyst-mediated functionalization of ring 'A' in salannin, a tetranortriterpene from *Azadirachta indica*

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Received (in Cambridge, UK) 30th May 2000, Accepted 20th July 2000
Published on the Web 22nd August 2000

Nocardia sp. quantitatively converts salannin **1** and 3-de-*O*-acetylsalannin **2** (C-seco limonoids) into 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin-1-en-3-one **10**, a novel and potentially bioactive compound with an α,β-unsaturated ketone moiety in ring 'A'. In order to establish the sequence of events involved in this transformation and the structural specificity of this bacterial system, several new derivatives of salannin **1** have been prepared. These studies have indicated that the transformation is initiated by deacetylation at C-3, followed by oxidation of the secondary hydroxy group to 3-keto, which appears to facilitate the elimination of the tigloyloxy/acetoxy group at C-1 with the formation of an olefinic linkage between C-1 and C-2.

The organism very efficiently transforms some of the derivatives of salannin into their corresponding compounds possessing an enone system in ring 'A', an essential pre-requisite for various biological activities. Some of the C-seco limonoids prepared in the present study, *viz*. **10**, 1,2-didehydro-1,3-dideoxy-3-oxosalannic acid **18**, 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]-20,21,22,23-tetrahydrosalannin-1-en-3-one **15** and 1,2-didehydro-1,3-dideoxy-3-oxosalannol **23** were hitherto not known.

Introduction

The phytochemical, biochemical and pharmacological properties of neem tree (*Azadirachta indica A. Juss*) constituents have attracted wide attention.[1–6]. They continue to interest structural and synthetic chemists for their complex array of functional groups, and the structural and stereochemical features present in many of them. The major class of compounds, which show marked insect antifeedant and growth-regulating activities, are the limonoids or tetranortriterpenes and their oxidized C-seco counterparts.[7–9] In fact, the C-seco limonoids are two to three orders of magnitude more active than the other limonoids and they possess high activity against herbivorous insects.[7–9]

Extensive studies carried out on the structure–activity relationships amongst limonoids clearly reveal that limonoids with an intact apoeuphol skeleton, a 14,15β-epoxide, and a reactive site such as either a 19–28 lactol bridge or a cyclohexenone 'A' ring are biologically very active.[10] Absence of these structural features results in reduced activity as exemplified by azadirachtin and salannin, the two major C-seco limonoids present in the neem seed; the former is known for its powerful antifeedant and insecticidal activity whereas the latter is devoid of any significant biological activity. The pronounced biological activity of some limonoids has prompted attempts to structurally modify limonoids such as salannin to enhance their activity.[11] Chemical modification of salannin and related compounds has been carried out and it was observed that antifeedant activity was increased by hydrogenation of the furan ring, replacement of the acetoxy group at C-3 with a methoxy group, saponification of the methyl ester at C-11, and hydrogenation of the (*E*)-2-methylbut-2-enoyl (tigloyl) group.[11] However, functionalization of ring 'A' in these compounds with an α,β-unsaturated ketone unit (an enone system) has not been attempted. It is known that C-seco limonoids with an enone system in ring 'A', as in nimbolide **5**, 28-deoxonimbolide **6** and gedunin **4** (Fig. 1), are potent cytotoxic and antimalarial agents.[12–14] The common feature primarily responsible for their biological activity, as has been shown, is the presence of an α,β-unsaturated ketone in ring 'A'.[15–17]

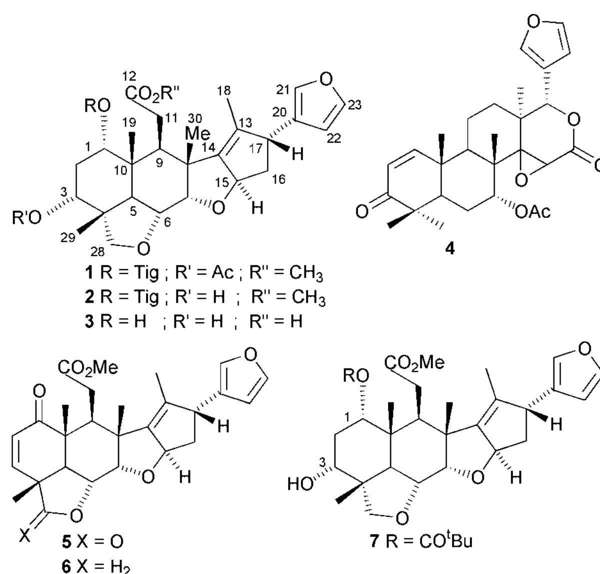

Fig. 1

In our earlier communication [18] we have reported the quantitative conversion of salannin **1** and 3-de-*O*-acetylsalannin **2** into a novel, structurally modified, and potentially bioactive compound, *viz*. 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]-salannin-1-en-3-one **10** using a growing culture of *Nocardia* sp. (see Scheme 1). In order to establish the sequence of events in the transformation and structural specificity of the organism, several new derivatives of salannin **1** were prepared. In the present paper we report the synthesis of new derivatives of salannin **1** and, more importantly, their efficient transformation using *Nocardia* sp. to C-seco limonoids with an enone system in ring 'A'. Some of the limonoids prepared in the present study were hitherto unknown.

DOI: 10.1039/b004260i

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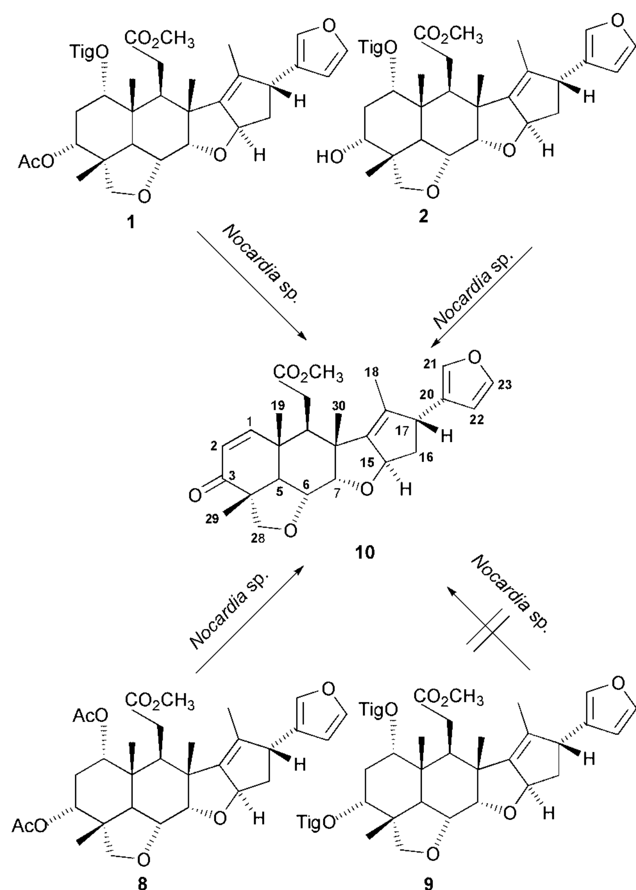
Results and discussion

In order to study the structural specificity of the organism in functionalizing ring 'A' to generate an enone system, the following derivatives of salannin were prepared.

Acetylation of 3-de-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **11** yielded 1-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **8**, the spectral data for which were in agreement with those reported.[11]



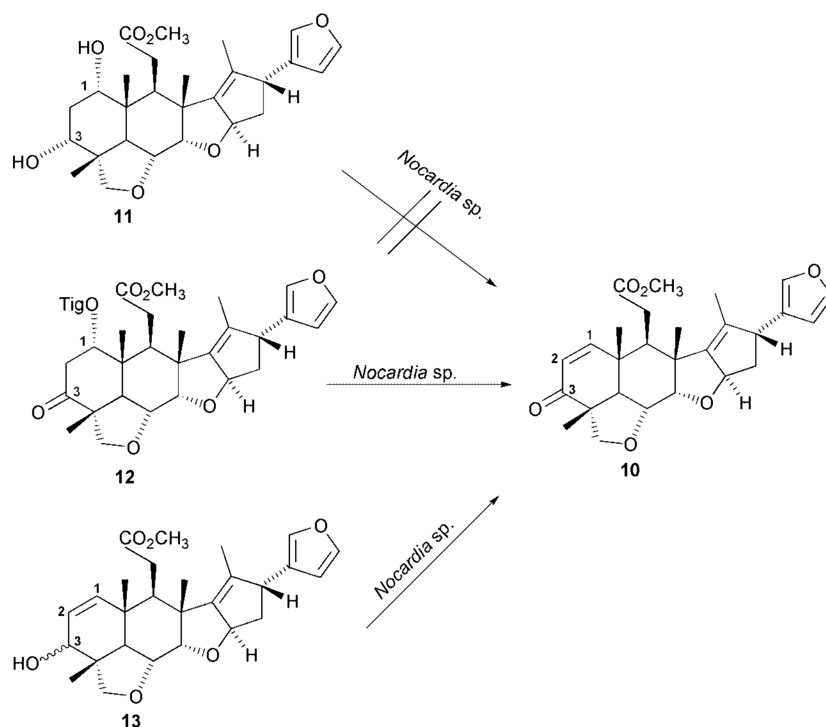
Scheme 1

Treatment of compound **11** with (*E*)-2-methylbut-2-enoyl chloride produced 3-de-*O*-acetyl-3-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **9**. The presence of the additional tigloyl group at C-3 was confirmed both from the mass and the ^{1}H NMR data of the compound.

Oxidation of 3-de-*O*-acetylsalannin **2** with PCC yielded 3-deacetoxy-3-oxosalannin **12** (see Scheme 2). The IR spectrum of **12** showed no absorption around 3300 cm^{-1}, indicating the absence of a hydroxy group. The absence of the signal from H-3, coupled with the deshielding of the signal from H-29, in the ^{1}H NMR spectrum of **12** clearly indicated the oxidation of the C-3 hydroxy group of **2**. The SEFT (spin echo Fourier transform) spectrum of the compound showed the presence of a signal at δ_C 207 assignable to that of the newly formed carbonyl functionality. The observed molecular-ion peak at *m*/*z* 552 (EI MS) further confirmed the oxidation of **2** to **12**.

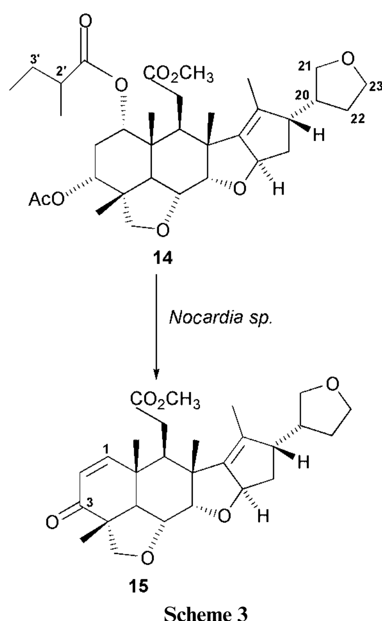
Hydrogenation of salannin **1** over palladium catalyst (10 atm) for a period of 6 hours yielded 2′,3′,20,21,22,23-hexahydrosalannin **14** (see Scheme 3). This was reflected in the ^{1}H NMR signals, wherein the downfield signal of the tigloyl group (H-3′) and the characteristic furan signals of H-20, H-21, H-22 and H-23 were missing, clearly indicating complete reduction to the hexahydro derivative.

Treatment of **10** with $NaBH_4$ in the presence of $CdCl_2$ resulted in the reduction of the C-3 ketone, forming the alcohol **13**. The IR spectrum showed an absorption at 3421 cm^{-1} indicating the presence of an alcohol. The upfield shift in the signals of H-1 (δ 5.41–5.44 in the ^{1}H NMR spectrum) and C-1 (δ_C 130.5 in the DEPT spectrum) coupled with the absence of a signal from C-3 carbonyl (DEPT spectrum), revealed the selective reduction of the enone to form the allylic alcohol **13**. The presence of the signal at δ 3.53 (3H, s) in the ^{1}H NMR spectrum and that of δ_C 173.9 in the DEPT spectrum of **13** indicated the methoxycarbonyl group to be intact. The ^{1}H NMR spectrum showed one set of peaks for the protons, indicating the formation of a single diastereoisomer of **13**. However, the stereochemistry of the alcohol at C-3 is not known. The molecular-ion peak (M$^+$) in the EI-MS was observed at *m*/*z* 454, an increase by two units as compared with that of **10**.



Scheme 2

An interesting feature observed on attempted acetylation (Ac_2O–Py) of salannic acid **3** was the formation of 3-*O*-acetyl-1-*epi*-salannic acid 12(1)-lactone **16**. The IR spectrum of **16** indicated the absence of the carboxylic group at C-11. The ^1H NMR spectrum of **16** was similar to that of salannic acid in the signals of the three-spin system H-5, H-6, H-7, the furan protons (H-21, H-22, H-23), the three methyls H_3-19, H_3-29 and H_3-30, and the AB system of H-11 and H-28. The two down-field signals at δ 4.41 and δ 4.82 indicated that the hydroxys at C-1 and C-3 had been functionalized. The difference in the chemical shift of these two signals, and the molecular-ion peak from the EI-MS data, ruled out the possibility of a diacetyl derivative. The only other plausible explanation was the formation of a lactone bridge between C-1 and C-12 under reaction conditions. The signal at δ_C 170.5 (DEPT spectrum), assignable to C-12, confirmed the presence of the lactone moiety in **16**. Further evidence was obtained by reducing the lactone to obtain a triol **19**. The stereochemistry at C-1 was determined from the multiplicity of the signal (^1H NMR spectrum of **16**) at δ 4.41, assignable to that of H-1. The appearance of H-1 as a double doublet with coupling constants of 12.9 and 4.5 Hz clearly indicates its α-orientation (α-axial). [The first doublet with a coupling constant of 12.9 Hz would be expected from a large vicinal (1,2-diaxial) coupling of H-1 (α-axial) with H^b-2 (β-axial). This doublet is further split by a small coupling (4.5 Hz) of H-1 (α-axial) with H^a-2 (α-equatorial) to give the observed double doublet]. As a necessary concomitant of the α-orientation of H-1, the C(1)–O linkage would be β-oriented giving rise to a *cis*-lactone between C-1 and C-12. Further, the reactions involved (reduction, acetylation and oxidation) in the formation of compounds **19**–**22** from the lactone **16** do not involve the C-1 carbon and hence the β-stereochemistry of C(1)–O (at C-1) is expected to be retained in all these compounds.

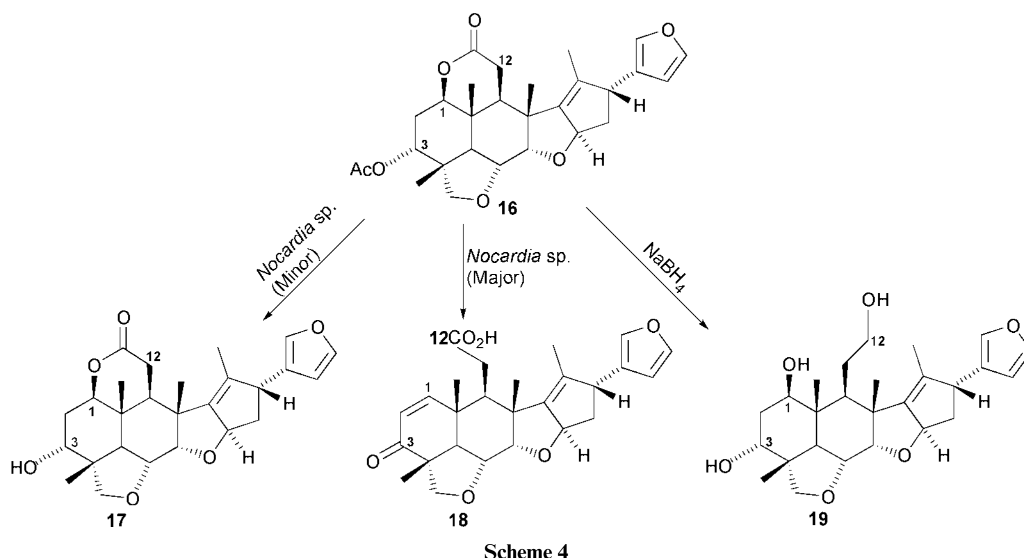


Scheme 3

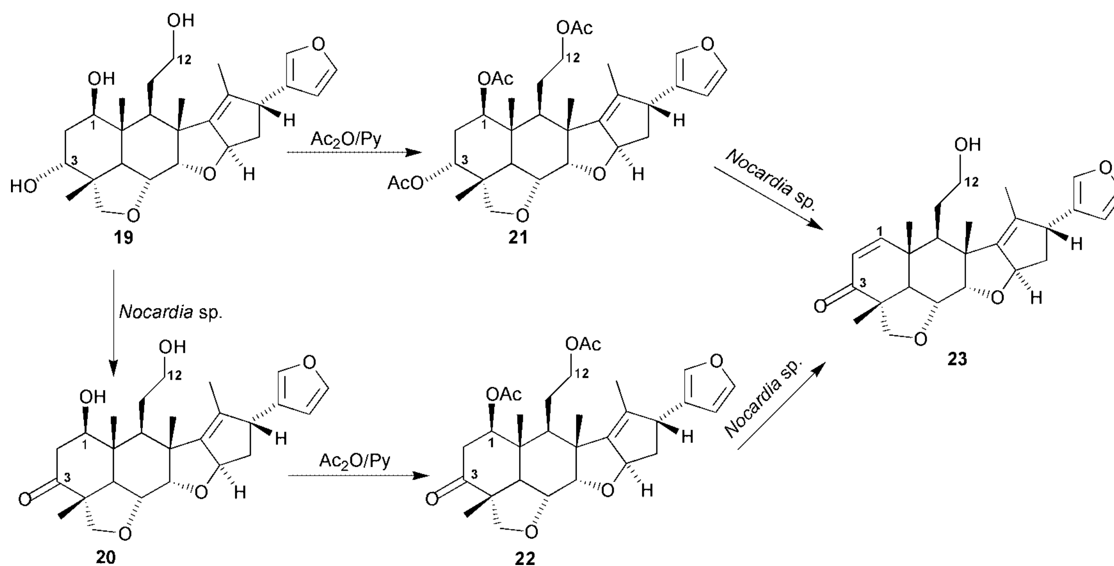
Reduction of the lactone **16** using $NaBH_4$ yielded the triol **19** (Scheme 4). The absorption at 3359 cm^{-1} in the IR spectrum indicated the presence of a hydroxy group. Further, the absence of absorption around 1720 cm^{-1} suggested the reduction of both the lactone [C(1)–C(12)] and C-3 acetate. The shielding of H-1 and H-3 signals ($\Delta\delta \approx 0.38$ and δ 0.94 ppm, respectively) in the ^1H NMR spectrum of **19**, revealed the absence of the C(1)–C(12) lactone bridge and the acetate moiety, respectively. The SEFT spectrum of **19** showed the presence of an additional methylene signal assignable to that of C-12. The chemical shift of the carbon at C-12 in the SEFT spectrum and that of its protons in the ^1H NMR spectrum revealed that it was attached to a hydroxy group. This triol **19** is unique among all the salannin analogues reported (both natural and synthetic) in that it has a reduced side-chain at C-9 as against the usual acetic acid side-chain. Thus, following the nomenclature of salannic acid, this compound **19** could be named salannol, as it is the C-1 epimer of the reduced side-chain analogue of salannic acid. However, this should not be confused with the salannol and its derivatives isolated by earlier workers.[19–21] A glimpse at the structure of the salannol (**7**; Fig. 1) reported earlier shows it to be a simple derivative of salannin. We feel that it is more appropriate to refer to the triol **19** as salannol. Furthermore, any derivative of salannin (either synthetic or naturally occurring) having the reduced side-chain alcohol at C-9 could be named following the basic skeleton of salannol.

Acetylation of our salannol **19** with Ac_2O–Py yielded the triacetate **21** (Scheme 5). The IR spectrum indicated the absence of any hydroxy group and the presence of carbonyl functionality (1735 cm^{-1}). The ^1H NMR spectrum of **21** indicated that the signals due to H-1 (δ 4.94), H-3 (δ 4.75) and H-12 (δ 3.56–3.76 and 4.08–4.15) were deshielded. This observation, coupled with the presence of three quaternary low-field signals in its SEFT spectrum, confirmed the acetylation of all three hydroxy groups. Further evidence obtained from the mass spectrum (EI) of **21** confirmed its structure to be 1,3,12-tri-*O*-acetylsalannol.

Acetylation of 3-deoxy-3-oxosalannol **20** using Ac_2O–Py yielded the diacetate 1,12-di-*O*-acetyl-3-deoxy-3-oxosalannol (3-deoxy-3-oxosalannol 1,12-diacetate) **22** (Scheme 5) as



Scheme 4



Scheme 5

Table 1

Substrate	Product(s) formed	Isolated yield (%)
1	**10**	95
2	**10**	97
8	**10**	91
12	**10**	97
13	**10**	40
14	**15**	95
16	**17**	38
	18	56
19	**20**	71 (≈90 Conversion)
21	**23**	70
22	**23**	95
24	**25**	89

evidenced from its IR, ^{1}H NMR, SEFT and mass spectral analyses.

Resting cells of *Nocardia* sp. very efficiently converted (see Experimental section and Table 1) salannin **1** into a metabolite (t_R 5.4 min, HPLC analyses) fully characterized (NMR, MS, HPLC) as 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin-1-en-3-one **10** (see Scheme 1). HPLC analyses revealed that the conversion was nearly quantitative (Table 1). A time-course study on the transformation of **1** carried out for a period of 8 hours indicated that during the early stages of incubation (2 h) almost equal quantities of **10** and another metabolite (t_R 6.7 min, HPLC analyses) were formed. However, with increasing incubation (4 h and thereafter) the metabolite with t_R 6.7 min disappeared with the concomitant formation of **10**. The compound with t_R 6.7 min was isolated pure as judged by TLC and HPLC analyses and identified as 3-deacetylsalannin **2** by comparison (NMR, MS, HPLC) with an authentic sample. The presence of **2** in the incubation mixture strongly supports our earlier observation that the conversion of **1** to **10** is initiated by selective hydrolysis of the acetate at C-3. This is further supported by the fact that resting cells of *Nocardia* sp. readily convert **2** into **10** (Scheme 1).

It is interesting to note that the organism efficiently converted 1-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **8** into a metabolite whose spectral characteristics (NMR, MS) matched well those of **10**, whereas 3-de-*O*-acetyl-3-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **9** was not accepted by the organism as a substrate (Scheme 1). These results suggest that the position of the two functionalities (acetate and tiglate) involved in the transformation seems to be critical in carrying out the functionalization of ring 'A'. So, it appears that a bulky substituent such as tigloyl at C-3 would prevent the organism carrying out the hydrolysis and subsequent oxidation to introduce an enone system in ring 'A', whereas, the presence of an acetyl group instead of tigloyl at C-1 as in **8** is a favorable structural disposition. It is surmised that the acetate functionality at C-3 plays a key role in the initiation of the transformation.

The next step in the transformation seems to be the oxidation of the secondary alcohol group of 3-de-*O*-acetylsalannin **2** to 3-deacetoxy-3-oxosalannin **12**. Both growing cultures and resting cells of *Nocardia* sp. efficiently transform **12** into **10**, indicating that **12** is indeed an intermediate in the pathway from **1** to **10** (Scheme 2). Surprisingly, 3-de-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **11** when incubated with the organism was not converted into **10** (Scheme 2). No other product was observed and the starting material was recovered. The fact that the organism does not accept **11** as a substrate rules out not only its possible intermediacy in the pathway from **1** to **10**, but also points out that the C-1 tigloyl group appears to remain intact until the C-3 acetyl group is hydrolyzed and oxidized as evidenced by the ready conversion of **2** and **12** into **10** (Schemes 1 and 2). Incubation of 3-de-*O*-acetyl-1,2-(didehydro-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin) **13** with *Nocardia* sp. resulted in the formation of **10**. However, the yield obtained in this case was low (40%), showing that **13** is not a good substrate for the organism (Scheme 2, Table 1). The unchanged material was isolated and found to be identical with the starting material **13**. This observation suggests that the organism prefers to oxidize the C-3 hydroxy group prior to formation of the 1,2-double bond.

Hydrogenation of the furan ring and the tigloyl group in salannin in known to enhance the antifeedant activity.[11] It would be interesting to find out if the organism could introduce an enone system in ring 'A' in 2′,3′,20,21,22,23-hexahydrosalannin **14** and, if so, whether such a transformation would result in the formation of a limonoid with not only a reduced furan ring, but also an α,β-unsaturated ketone unit in ring 'A'. One would expect such a compound to be biologically much more active than the parent compound **14**. It was found that incubation of **14** with the resting cells of *Nocardia* sp. yielded 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]-20,21,22,23-tetrahydrosalannin-1-en-3-one **15** as the only metabolite (Scheme 3, Table 1). The strong absorbance at 1685 cm^{-1} in the IR spectrum of the metabolite **15** indicated the presence of a conjugated carbonyl functionality. The ^{1}H NMR spectrum exhibited the characteristic doublets (δ 6.74 and 5.84) coupled to each other (J = 10 Hz), further supporting the presence of an

α,β-unsaturated ketone in **15**. The signals appearing at δ_C 154.6, 127.3 and 200.9 in the DEPT spectrum of **15** further confirmed the presence of an α,β-unsaturated ketone. Since the remaining signals were similar to those of the parent compound **14**, the functionalization should have occurred in ring 'A', leaving the rest of the molecule intact.

Incubation of 3-*O*-acetyl-1-*epi*-salannic acid 12(1)-lactone **16** with the resting cells of *Nocardia* sp. yielded two metabolites, one neutral and the other acidic (Scheme 4, Table 1). The neutral metabolite showed in its IR spectrum an absorption for an hydroxy group at 3414 cm^{-1}. The ^1H NMR spectrum of this metabolite showed the absence of the methyl signals from the acetate moiety at C-3. Also there was a considerable upfield shift of the signal from the proton at C-3, again indicating the absence of the acetate moiety. This was very much in agreement with the EI-MS of this metabolite wherein a peak at *m/z* 440 (molecular-ion peak) was observed. Thus the metabolite was identified as 1-*epi*-salannic acid 12(1)-lactone **17**.

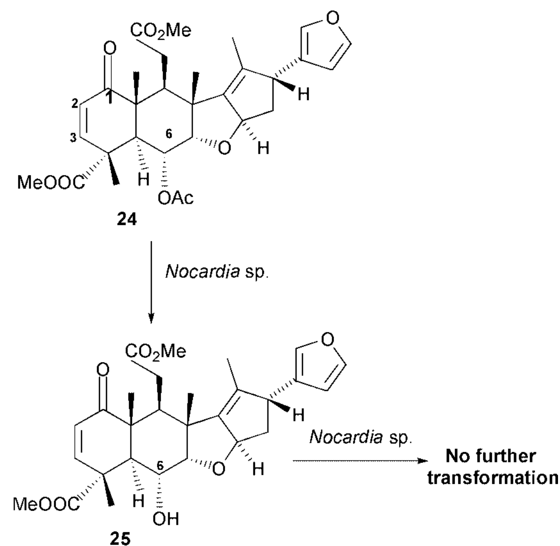
The IR spectrum of the acidic metabolite showed absorptions at 3500 cm^{-1} (br) (CO_2H) and 1684 cm^{-1} (α,β-unsaturated carbonyl functionality). The appearance of two low-field doublets (δ 6.76 and 5.78) coupling to each other in the ^1H NMR spectrum of this metabolite clearly suggested the presence of an enone system. This was further supported by the SEFT spectrum wherein the signals for the enone system were observed (δ_C 154.5, 127.5 and 201.02). Since the rest of the signals were similar to those of the parent compound **16** the changes can only be confined to ring 'A'. Thus this metabolite was identified as 1,2-didehydro-1,3-dideoxy-3-oxosalannic acid **18**. Further evidence obtained from the high-resolution mass data (EI-HRMS) indicated a molecular formula of $C_{26}H_{30}O_6$ and confirmed the structure assigned. The isolation of 1-*epi*-salannic acid 12(1)-lactone **17** from **16** clearly supports our earlier observation that enone formation is initiated through deacetylation at the C-3 position. The removal of the proton at C-2 with concomitant opening of the lactone generates the C-1–C-2 olefinic linkage. As observed, this process does not occur readily, thereby suggesting that the lactone functionality is not a good leaving group in comparison with other esters tested.

Prolonged incubation (2–3 days) of salannol **19** with the growing culture of *Nocardia* sp. (see Experimental section) yielded only one major metabolite (Scheme 5, Table 1). The IR spectrum of this metabolite showed absorptions at 3412 and 1713 cm^{-1}, indicating the presence of a hydroxy and a carbonyl functionality, respectively. The absence of one of the two secondary hydroxy groups, as observed from the integration of the multiplet at δ 4.06–4.16, in the ^1H NMR spectrum clearly indicated the oxidation of either C-1 or C-3. The anisotropic deshielding of the signals from H_3-28 and H_3-29 revealed that the oxidation had occurred at C-3. Further evidence from the mass spectrum (EI-HRMS) of this metabolite confirmed it to be 3-deoxy-3-oxosalannol **20**, (Scheme 5).

Incubation of 1,3,12-tri-*O*-acetylsalannol (salannol triacetate) **21** with the growing culture of *Nocardia* sp. yielded a major metabolite (Scheme 5, Table 1). The IR spectrum of this metabolite showed absorptions at 1683 and 3463 cm^{-1}, indicating the presence of an α,β-unsaturated ketone and an alcohol, respectively. The ^1H NMR spectrum of the product showed two low-field doublets in the region of δ 6.98 and δ 5.86 coupling to each other ($J = 10$ Hz), suggesting the presence of a conjugated olefinic system. The methyl signals from the acetate groups were missing. The signals at δ_C 155.86, δ_C 127.12 and δ_C 201.36 in the SEFT spectrum of this metabolite confirmed the presence of an α,β-unsaturated ketone moiety. The upfield shift in the multiplets arising from H-12 (δ 3.55–3.69) indicated that the C-12 acetate had been hydrolyzed to the corresponding alcohol. As the remaining signals were similar to those of the parent compound **21**, the α,β-unsaturated ketone must be part of ring 'A'. From the spectral analyses, this metabolite was identified as a new compound, 1,2-didehydro-1,3-dideoxy-3-oxosalannol **23**.

Incubation of 1,12-di-*O*-acetyl-3-deoxy-3-oxosalannol **22** with the growing culture of *Nocardia* sp. (see Experimental section) yielded exclusively one metabolite fully characterized on the basis of various spectral analyses (IR, NMR, MS) as compound **23** (Scheme 5, Table 1). The ready formation of **23**, a compound with an enone system in ring 'A', from both **21** and **22** not only points out the ability of the organism to carry out regiospecific oxidation at C-3 as seen in the conversion of **19** into **20**, but also further substantiates the proposed sequence of events leading to the formation of **10** from **1**. These transformations clearly suggest that the elimination of the C-1 substituent (acetoxy/tigloyloxy) with the formation of an olefinic linkage between C-1 and C-2 takes place in a single step after the secondary hydroxy group at C-3 is oxidized.

It is interesting to note that nimbin **24**, a biogenetic precursor to salannin **1**, when incubated with the resting cells of *Nocardia* sp., was efficiently transformed (Table 1) into only one metabolite, identified as 6-de-*O*-acetylnimbin **25** (Scheme 6). This



Scheme 6

metabolite did not undergo further transformation. Nimbin **24** already has an enone system in ring 'A' and it appears that one of the characteristic features of this organism is that it fails to further metabolize limonoids with an enone system in ring 'A', as exemplified with compounds **10**, **15**, **18** and **23**. It is quite possible that the conversion of nimbin **24** to 6-de-*O*-acetylnimbin **25** could have been catalyzed by a non-specific esterase.

The present study documents for the first time the structural modification of some of the C-seco limonoids using biocatalysts. In fact, some of the C-seco limonoids prepared with the novel α,β-unsaturated ketone moiety in the ring 'A' were hitherto unknown and could prove to be potential cytotoxic and antimalarial agents. The study also demonstrates the unique ability of *Nocardia* sp. to functionalize ring 'A' by introducing an enone system in salannin **1** and some of its derivatives (**14**, **16**, **21**, **22**) thereby demonstrating its wide substrate specificity and structural flexibility. Although hypothetically it is possible to introduce a 2-en-1-one system into ring 'A' of salannin and its derivatives, the organism exhibits a rigid specificity in oxidizing the C-3 over C-1 position, indicating that a 1-en-3-one is preferred over 2-en-1-one system in ring 'A' of these compounds.

Experimental

Neem seeds were collected in Tamil Nadu, India during the month of December. IR spectra were recorded using a JASCO

FT-IR-410 spectrometer. Spectra were recorded as thin films of solutions in chloroform. NMR studies were carried out on a JEOL JNM-LA 300 FT, a Bruker AMX 400 MHz, or a Bruker DRX 500 MHz instrument for samples in $CDCl_3$ with TMS as internal standard. *J*-Values are quoted in Hz and chemical shifts are reported relative to TMS. Mass spectra were determined using a JEOL JMS-DX 303 spectrometer in the EI mode of ionization. Optical rotations were measured on a JASCO DIP-370 polarimeter; $[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. TLC analyses were performed on silica gel G plates (0.5 mm) developed with EtOAc–hexane (75:25) as the solvent system. Compounds were visualized by spraying the plates with vanillin–H_2SO_4 [3% vanillin in methanol (w/v) and 1% conc. H_2SO_4 (v/v)] followed by heating them to 140 °C. HPLC analysis was carried out on a Shimadzu CR 7A instrument using an ODS reversed-phase column (μ-bondapak) with CH_3OH–water (60:40) as the solvent system. Compounds were detected using a Waters UV-variable detector set to 238 nm. Flow rate was maintained at 1 ml min^{-1}. Extracts were dried over anhydrous sodium sulfate.

Materials

Isolation of salannin, 3-deacetylsalannin and nimbin. Salannin **1**, 3-de-*O*-acetylsalannin **2** and nimbin **24** were isolated from the methanolic extract of neem seed kernels of *Azadirachta indica*, following Nakanishi′s procedure.[22] Fractions containing salannin, de-*O*-acetylsalannin and nimbin were subjected to column chromatography over silica gel using hexane–EtOAc as the solvent system. Further purification was carried out by recrystallization from hexane–EtOAc.

Salannic acid **3**, 3-de-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin**11**,1-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]-salannin **8** and 2′,3′,20,21,22,23-hexahydrosalannin **14** were prepared by the method of Yamasaki and Klocke.[11]

Synthesis of 3-de-*O*-acetyl-3-*O*-[(*E*)-2-methylbut-2-enoyl]-salannin 9. To a stirred solution of 3-de-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **11** (100 mg, 0.2 mmol) in dry CH_2Cl_2 (≈5 ml) were added (*E*)-2-methylbut-2-enoyl chloride (71 mg, 3 eq.), pyridine and a catalytic amount of 4-(dimethylamino)pyridine (DMAP). The mixture was stirred at RT for 48 h, poured into saturated aq. sodium bicarbonate, and extracted three times with 10 ml portions of CH_2Cl_2. The organic layer was then washed successively with water and brine, and dried over anhydrous sodium sulfate. It was then concentrated and passed through a pad of silica gel, using $CHCl_3$–CH_3CN as the solvent system, to obtain title compound **9** (74 mg, 55%), mp 136–138 °C (from acetone); $[\alpha]_D^{25}$ +83 (*c* 1, $CHCl_3$); δ_H (300 MHz) 0.94 (3H, s, H_3-19), 1.84–2.41 (6H, m, H_2-2, -11, -16), 1.21–2.00 (18H, m, H_3-4′, -5′, -4″, -5″, -29, -30), 1.67 (3H, s, H_3-18), 2.65 (1H, d, *J* 12.6, H-5), 2.78 (1H, d, *J* 4.5, H-9), 3.57–3.71 (3H, m, H-17, H_2-28), 4.00 (1H, dd, *J* 12.6 and 3, H-6), 4.18 (1H, d, *J* 3, H-7), 4.79 (1H, d, *J* 2.4, H-1), 4.95 (1H, s, H-3), 5.44 (1H, s, H-15), 6.30 (1H, s, H-22), 6.85–6.99 (2H, m, H-3′, -3″), 7.27 (1H, s, H-21), 7.33 (1H, s, H-23); δ_C (100 MHz) 11.92 (C-5′), 12.84 (C-5″), 12.95 (C-18), 14.22 (C-4′), 15.04 (C-4″), 16.16 (C-19), 16.82 (C-30), 19.59 (C-29), 30.71 (C-11), 39.89 (C-9), 41.39 (C-10), 41.49 (C-16), 42.85 (C-2), 48.76 (C-4), 49.01 (C-8), 49.43 (C-5), 49.43 (C-17), 71.45 (C-1), 72.56 (C-3), 72.67 (C-6), 77.68 (C-28), 85.69 (C-7), 87.86 (C-15), 110.56 (C-22), 127.05 (C-20), 129.06 (C-2′), 129.07 (C-2″), 134.81 (C-13), 136. 61 (C-3″), 137.27 (C-3′), 138.83 (C-21), 142.87 (C-23), 146.63 (C-14), 166.79 (C-1″), 167.17 (C-1′), 174.05 (C-12). *m*/*z* 635 ($M^+ - H$), 620 [($M^+ - H$) − CH_3], 568.

Synthesis of 3-de-*O*-acetyl-1,2-didehydro-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin 13. To a stirred suspension of 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin-1-en-3-one **10** (300 mg, 0.7 mmol) and $CdCl_2$ (1 eq., 121 mg) in propan-2-ol (≈10 ml) was added $NaBH_4$ (1.2 eq., 30 mg) portionwise. After completion of the reaction (TLC, 15 h), the solvent was evaporated off, and the residue was stirred with mild acid and then extracted with chloroform (thrice with 20 ml portions). The organic layer was then dried, evaporated and then passed through a pad of silica gel, using $CHCl_3$–CH_3CN as the solvent system, to obtain pure compound **13** (256 mg, 85%), mp 132–134 °C (from MeOH); $[\alpha]_D^{25}$ +124 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 3421, 1733; δ_H (300 MHz) 1.05 (3H, s, H_3-19), 1.10 (3H, s, H_3-29), 1.31 (3H, s, H_3-30), 1.69 (3H, d, *J* 1.5, H_3-18), 2.09–2.19 (3H, m, H-9, H_2-16), 2.24 (1H, d, *J* 12, H-5), 2.37 (1H, dd, *J* 15.5, H^b-11), 2.55 (1H, dd, *J* 15 and 7, H^a-11), 3.53 (3H, s, CO_2Me), 3.65 (1H, d, *J* 8, H-17), 3.79 (1H, d, *J* 8, H^b-28), 3.97 (1H, d, *J* 8, H^a-28), 4.09 (1H, dd, *J* 12 and 3.3, H-6), 4.27 (1H, d, *J* 3.3, H-7), 4.35 (1H, br s, H-3), 5.41–5.44 (2H, m, H-1, -15), 5.68 (1H, dd, *J* 10 and 2.4, H-2), 6.23 (1H, s, H-22), 7.21 (1H, s, H-21), 7.32 (1H, t, *J* 1.5, H-23); δ_C (75 MHz) 12.8 (C-18), 14.7 (C-30), 16.7 (C-19), 17.7 (C-29), 31.4 (C-11), 38.6 (C-10), 41.5 (C-16), 43.0 (C-4), 46.9 (C-9), 49.4 (C-8), 49.5 (C-5), 49.5 (C-17), 51.8 (CO_2Me), 73.3 (C-6), 83.4 (C-28), 87.1 (C-7), 87.9 (C-15), 110.3 (C-22), 126.9 (C-20), 130.5 (C-1), 134.6 (C-13), 135.5 (C-2), 138.6 (C-21), 142.9 (C-23), 173.9 (C-12); *m*/*z* 454 (M^+), 423 ($M^+ - OMe$), 392, 259.

Synthesis of 3-*O*-acetyl-1-*epi*-salannic acid 12(1)-lactone 16. Salannic acid (1 g, 2.18 mmol) was dissolved in dry acetic anhydride (10 ml) containing pyridine (2 ml). A catalytic amount of DMAP was added and the solution was stirred for 10 h at RT. The reaction mixture was then diluted with excess of water and extracted thrice with CH_2Cl_2. The organic layer was thoroughly washed succesively with saturated aq. sodium bicarbonate, water and brine. It was then dried over anhydrous sodium sulfate, concentrated and passed through a pad of silica gel, using $CHCl_3$–CH_3CN as the solvent system, to obtain 3-*O*-acetyl-1-*epi*-salannic acid 12(1)-lactone **16** (945 mg, 90%), mp 222–224 °C (from acetone); $[\alpha]_D^{25}$ +75 (*c* 1, $CHCl_3$): ν_{max}/cm^{-1} 1735; δ_H (300 MHz) 1.23 (3H, s, H_3-30), 1.29 (3H, s, H_3-29), 1.32 (3H, s, H_3-19), 1.91–2.57 (7H, m, H_2-2, H-9, H_2-11, H_2-16), 2.06 (3H, s, acetate), 2.62 (1H, d, *J* 12, H-5), 3.61 (1H, d, *J* 7.5, H^b-28), 3.72 (1H, d, *J* 8.7, H-17), 3.81 (1H, d, *J* 7.5, H^b-28), 4.07 (1H, dd, *J* 12 and 3.3, H-6), 4.31 (1H, d, *J* 3.3, H-7), 4.41 (1H, dd, *J* 12.9 and 4.5, H-1), 4.82 (1H, dd, *J* 9.9 and 6.6, H-3), 5.35–5.38 (1H, m, H-15), 6.13 (1H, s, H-22), 7.16 (1H, s, H-21), 7.36 (1H, d, *J* 1.5, H-23); δ_C (100 MHz) 13.07 (C-18), 17.45 (C-19), 18.98 (C-29), 20.67 (C-30), 22.16 (acetate), 28.73 (C-2), 32.39 (C-10), 33.43 (C-11), 40.93 (C-9), 41.66 (C-16), 42.37 (C-4), 47.74 (C-8), 49.42 (C-17), 49.99 (C-5), 73.11 (C-6), 75.27 (C-3), 77.87 (C-28), 85.72 (C-7), 87.46 (C-15), 88.25 (C-1), 110.01 (C-22), 126.51 (C-20), 135.55 (C-13), 138.43 (C-21), 143.48 (C-23), 145.57 (C-14), 170.54 (C-12), 170.61 (acetate); *m*/*z* 482 (M^+), 464.

Syntheses of our salannol 19 {8α-(3-furyl)-2a,4,5,5a,6,6a,8,9,9aα,10aβ,10bβ,10cα-dodecahydro-6β-(2-hydroxyethyl)-2aβ,5aβ,6aβ,7-tetramethyl-2*H*,3*H*-cyclopenta[*d*′]naphtho[1,8-*bc*:2,3-*b*′]difuran-3α,5β-diol}. 3-*O*-Acetyl-1-*epi*-salannic acid 12(1)-lactone **16** (500 mg, 1 mmol) was dissolved in propan-2-ol and $NaBH_4$ was added portionwise with stirring. After 6 h, propan-2-ol was removed, water was added, and the resultant solution extracted with $CHCl_3$ thrice. The organic layer was dried and concentrated to obtain pure triol **19** in quantitative yield, mp 265–267 °C (from MeOH); $[\alpha]_D^{25}$ +139 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 3359; δ_H (300 MHz) 0.87 (3H, s, H_3-30), 1.09 (3H, s, H_3-29), 1.29 (3H, s, H_3-19), 1.66–1.71 (1H, m, H^b-11), 1.71 (3H, d, *J* 1.5, H_3-18), 2.09–2.29 (6H, m, H_2-2, H^a-11, H-9, H_2-16), 2.64 (1H, d, *J* 13.5, H-5), 3.48–3.75 (5H, m, H_2-12, H-17, H_2-28), 3.88 (1H, s, H-3), 3.99–4.06 (2H, m, H-1, H-6), 4.22 (1H, d, *J* 3.3, H-7), 5.48–5.53 (1H, m, H-15), 6.22 (1H, s, H-22), 7.21 (1H, s, H-21), 7.34 (1H, t, *J* 1.5, H-23); δ_C (75 MHz) 12.91 (C-18), 14.97 (C-30), 17.18 (C-19), 19.13 (C-29), 27.68 (C-2),

30.75 (C-11), 37.80 (C-10), 38.69 (C-9), 40.69 (C-5), 41.34 (C-16), 44.31 (C-4), 49.26 (C-17), 49.35 (C-8), 62.33 (C-12), 71.63 (C-6), 72.82 (C-3), 72.96 (C-1), 77.69 (C-28), 86.19 (C-7), 87.73 (C-15), 110.15 (C-22), 127.15 (C-20), 127.81 (C-14), 133.04 (C-13), 138.43 (C-21), 143.30 (C-23); *m/z* 444 (M^+), 429 ($M^+ - CH_3$), 411 (429 − H_2O), 393 (411 − H_2O).

Synthesis of 1,3,12-tri-*O*-acetylsalannol 21. Our salannol **19** (400 mg, 0.9 mmol) was acetylated using dry Ac_2O–Py with a catalytic amount of DMAP. The reaction mixture was stirred at RT for 12 h, diluted with water and extracted with CH_2Cl_2. The organic layer was washed well and successively with aq. sodium bicarbonate, water, and finally with brine. It was then concentrated, and filtered through a small pad of silica gel, using $CHCl_3$–CH_3CN as the solvent system, to obtain pure triacetate **21** (440 mg, 86%), mp 104–106 °C (from acetone); $[\alpha]_D^{25}$ +105 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 1735; δ_H (300 MHz) 0.97 (3H, s, H_3-30), 1.21 (3H, s, H_3-29), 1.34 (3H, s, H_3-19), 1.54–2.31 (6H, m, H_2-2, -11, -16), 1.81 (3H, d, *J* 1.2, H_3-18), 1.99 (3H, s, acetate), 2.04 (3H, s, acetate), 2.07 (3H, s, acetate), 2.67 (1H, d, *J* 12, H-5), 3.56–3.76 (4H, m, H^a-12, H-17, H_2-28), 3.97 (1H, dd, *J* 12 and 3.3, H-6), 4.08–4.15 (2H, m, H-7, H^b-12), 4.75 (1H, s, H-3), 4.94 (1H, t, *J* 3, H-1), 5.31 (1H, s, H-15), 6.18 (1H, s, H-22), 7.24 (1H, s, H-21), 7.36 (1H, t, *J* 1.5, H-23); δ_C (100 MHz) 13.67 (C-18), 15.06 (C-30), 17.10 (C-19), 19.28 (C-28), 19.66 (acetate), 21.16 (acetate), 21.28 (acetate), 25.35 (C-11), 27.65 (C-2), 39.05 (C-9), 39.73 (C-5), 40.45 (C-10), 41.61 (C-16), 47.77 (C-4), 48.82 (C-8), 49.49 (C-17), 63.95 (C-12), 71.42 (C-6), 72.47 (C-3), 72.65 (C-1), 77.69 (C-28), 85.72 (C-7), 87.63 (C-15), 110.23 (C-22), 127.05 (C-20), 134.17 (C-13), 138.49 (C-21), 143.36 (C-23), 147.64 (C-14), 169.89 (acetate), 170.41 (acetate), 171.12 (acetate); *m/z* 570 (M^+), 528, 510, 435, 213.

Synthesis of 1,12-di-*O*-acetyl-3-deoxy-3-oxosalannol 22. 3-Deoxy-3-oxosalannol **20** (50 mg, 0.1 mmol) was stirred in dry Ac_2O (1 ml) and pyridine (0.2 ml) with a catalytic amount DMAP. After 2 h the reaction mixture was diluted with excess of water and extracted thrice with CH_2Cl_2. The organic layer was washed well and successively with aq. sodium bicarbonate, water, and finally with brine. It was then concentrated, and passed through a small pad of silica gel, using $CHCl_3$–CH_3CN as the solvent system, to obtain pure compound **22** (54 mg, 91%), mp 127–130 °C (from acetone); $[\alpha]_D^{25}$ +128 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 1737; δ_H (500 MHz) 1.21 (3H, s, H_3-30), 1.36 (3H, s, H_3-29), 1.41 (3H, s, H_3-19), 1.81 (3H, d, *J* 1.5, H-18), 1.98 (3H, s, acetate), 2.08 (3H, s, acetate), 2.07–2.29 (3H, m, H-9, H_2-16), 2.43 (1H, dd, *J* 15.7 and 2.7, H^a-2), 2.69 (1H, d, *J* 12, H-5), 3.09 (1H, dd, *J* 15.7 and 3.7, H^b-2), 3.66–3.71 (3H, m, H^a-12, H-17, H^b-28) [including 3.70 (1H, d, *J* 8.1, H^b-28)], 4.02 (1H, d, *J* 8.1, H^a-28), 4.08–4.16 (3H, m, H^b-12, H-6, -7) [including 4.09 (1H, dd, *J* 12 and 3, H-6), 4.15 (1H, d, *J* 3, H-7)], 5.15 (1H, t, *J* 3.2, H-1), 5.23–5.26 (1H, m, H-15), 6.15 (1H, s, H-22), 7.22 (1H, s, H-21), 7.35 (1H, t, *J* 1.6, H-23); δ_C (100 MHz) 13.58 (C-18), 14.94 (C-29), 16.81 (C-30), 21.05 (acetate), 21.05 (acetate), 25.71 (C-11), 38.76 (C-9), 40.49 (C-10), 41.37 (C-16), 46.03 (C-5), 49.05 (C-8), 49.05 (C-4), 49.39 (C-17), 52.81 (C-2), 63.49 (C-12), 72.14 (C-6), 76.01 (C-28), 77.94 (C-1), 85.39 (C-7), 87.52 (C-15), 110.05 (C-22), 126.79 (C-20), 134.61 (C-13), 143.29 (C-23), 147.06 (C-14), 169.47 (acetate), 169.47 (acetate), 207.51 (C-3); *m/z* 526 (M^+), 495, 451, 213.

Microorganism and growth media

Nocardia sp. used in the present study was isolated from soil. Based on various morphological, cultural and biochemical characteristics the organism was identified as belonging to the genus *Nocardia*.

Nocardia sp. was maintained at 3 °C on nutrient agar slants. A starter culture of the organism was prepared by transferring it from the nutrient agar slant to a 100 ml sterilized liquid mineral salts medium[23] (pH 7.2) containing 0.05% yeast extract and 0.25% glucose and incubating this on rotary shaker (220 rpm) at 29–30 °C for 24 h.

General procedure for biotransformation

Experiments carried out using growing culture of *Nocardia* sp. Biotransformation experiments were conducted in 500 ml Erlenmeyer flasks containing 100 ml of sterile modified mineral salts medium[23] (pH 7.2) containing 0.05% yeast extract and 0.25% glucose. The flasks were inoculated from a 24 h-old culture (5 ml, $A_{660} \approx 1.4$) and incubated on a rotary shaker (220 rpm). After 24 h of growth, various substrates [**19** (1.13 mmol l^{-1}, 100 mg)/**21** (0.88 mmol l^{-1}, 100 mg)/**22** (0.95 mmol l^{-1}, 50 mg)] were added in methanol–acetone and the incubation continued. The transformation monitored by TLC was stopped at appropriate time intervals (2–3 days) and the broth extracted with an equal volume of ethyl acetate (× 2). The organic phase was dried over sodium sulfate and concentrated *in vacuo*. Further purification was carried out over silica gel, using $CHCl_3$–CH_3CN as the solvent system, to yield pure, 3-deoxy-3-oxosalannol **20** (71 mg, 71%, ≈ 90% conversion) from **19**; 1,2-didehydro-1,3-dideoxy-3-oxosalannol **23** [(52 mg, 70%) from **21**; (40 mg, 95%) from **22**] (Table 1).

Resting-cells experiment. Flasks containing 100 ml of sterile modified mineral salts medium[23] (pH 7.2) containing 0.05% yeast extract and 0.25% glucose were inoculated from a 24 h-old culture (5 ml; $A_{660} \approx 1.4$) and incubated on a rotary shaker at 29–30 °C for 36 h. At the end of the incubation period the cells were harvested by centrifugation (5000 ***g***; 20 min), washed well with phosphate buffer (10 mM; pH 7.2) and suspended in the same buffer (1 g wet weight in 20 ml). To this cell suspension were added various substrates, salannin **1** (0.17 mmol, 100 mg), 2′,3′,20,21,22,23-hexahydrosalannin **14** (0.17 mmol, 100 mg), 1-*O*-acetyl-1-de-*O*-[(*E*)-2-methylbut-2-enoyl]salannin **8** (90 µmol, 50 mg), 3-de-*O*-acetyl-3-*O*-[(*E*)-2-methylbut-2-enoyl]-salannin **9** (78.6 µmol, 50 mg), 3-de-*O*-acetyl-1,2-didehydro-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin **13** (0.22 mmol, 100 mg), 3-*O*-acetyl-1-*epi*-salannic acid 12(1)-lactone **16** (0.21 mmol, 100 mg), nimbin **24** (92.6 µmol, 50 mg), in acetone (0.2 ml) and incubated on a rotary shaker (220 rpm) at 29–30 °C. Aliquots were taken at regular time intervals and the progress of the reaction monitored by TLC/HPLC. The incubation was stopped at the appropriate time (12–15 h), the cell suspension centrifuged (5000 ***g***; 20 min) and the supernatant extracted with ethyl acetate (thrice with 20 ml). The cells were separately extracted with ethyl acetate, the organic layers pooled, dried over sodium sulfate, and the solvent removed under reduced pressure. The residue was passed through a pad of silica gel, using $CHCl_3$–CH_3CN as the solvent system, to obtain pure 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]salannin-1-en-3-one **10** [(72 mg, 95%) from **1** (37 mg, 91%) from **8** (40 mg, 40%) from **13**]; 3-deacetoxy-1-de[(*E*)-2-methylbut-2-enoyloxy]-20,21,22,23-tetrahydrosalannin-1-en-3-one **15** (72 mg, 95%) from **14**; 1,2-didehydro-1,3-dideoxy-3-oxosalannic acid **18** (48 mg, 56% from a mixture of **17** and **18**) from **16**; 1-*epi*-salannic acid 12(1)-lactone **17** (32 mg, 38% from a mixture of **17** and **18**) from **16**; 6-de-*O*-acetylnimbin **25** (41 mg, 89%) from nimbin **24**.

3-Deacetoxy-1-de[(E)-2-methylbut-2-enoyloxy]salannin-1-en-3-one ***10***. The data for this metabolite have been reported earlier.[18]

3-Deacetoxy-1-de[(E)-2-methylbut-2-enoyloxy]-20,21,22,23-tetrahydrosalannin-1-en-3-one ***15***. Mp 68–70 °C (from acetone); $[\alpha]_D^{25}$ +121 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 1735, 1685; δ_H (300 MHz) 1.19 (3H, s, H_3-19), 1.29 (3H, s, H_3-30), 1.33 (3H, s, H_3-29), 1.40–2.00 (4H, m, H_3-18, H-20), 1.90–2.36 (6H, m, H-9, H_2-16, H-17, H_2-22), 2.41–2.65 (2H, m, H_2-11), 2.69 (1H, d, *J* 12.3, H-5), 3.32–3.60 (1H, m, H-21), 3.66 (3H, s, CO_2Me),

3.69–4.03 (3H, m, H-23, H_2-28), 4.12 (1H, dd, *J* 12.3 and 3.6, H-6), 4.22 (1H, d, *J* 3.6, H-7), 5.25–5.28 (1H, m, H-15), 5.84 (1H, d, *J* 10, H-2), 6.74 (1H, d, *J* 10, H-1); δ_C (75 MHz) 13.4 (C-18), 15.4 (C-30), 17.5 (C-19), 18.2 (C-29), 31.2 (C-11), 37.9 (C-22), 38.4 (C-16), 39.8 (C-10), 42.6 (C-20), 44.8 (C-9), 48.8 (C-4), 49.3 (C-17), 49.4 (C-5), 49.7 (C-8), 51.9 (CO_2Me), 67.8 (C-21), 72.3 (C-23), 72.4 (C-6), 76.9 (C-28), 85.8 (C-7), 87.5 (C-15), 127.3 (C-2), 135.5 (C-13), 146.0 (C-14), 154.6 (C-1), 173.2 (C-12), 200.9 (C-3); *m/z* 456 (M^+), 425 ($M^+ - OMe$), 384 (EI-HRMS): Found: M^+, 456.251750. $C_{27}H_{36}O_6$ requires *M*, 456.251189.

1-epi-Salannic acid 12(1)-lactone ***17***. Mp 176–178 °C (from acetone); $[\alpha]_D^{25}$ +40 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 3414, 1727; δ_H (300 MHz) 1.18 (3H, s, H_3-30), 1.19 (3H, s, H_3-29), 1.31 (3H, s, H_3-19), 1.75 (3H, d, *J* 1.5, H_3-18), 1.91–2.56 (7H, H_2-2, H-9, H_2-11, -16), 2.63 (1H, d, *J* 12.3, H-5), 3.68–3.74 (2H, H-17, H^a-28), 3.98–4.09 (2H, H-3, -6), 4.14 (1H, d, *J* 8, H^b-28), 4.31–4.37 (2H, m, H-1, -7), 5.33–5.38 (1H, m, H-15), 6.12 (1H, s, H-22), 7.15 (1H, s, H-21), 7.35 (1H, t, *J* 1.5, H-23); δ_C (100 MHz) 12.97 (C-18), 17.37 (C-19), 18.93 (C-30), 22.69 (C-29), 28.73 (C-2), 32.47 (C-10), 37.35 (C-11), 41.03 (C-9), 41.54 (C-16), 42.95 (C-4), 47.72 (C-8), 49.29 (C-17), 49.41 (C-5), 73.02 (C-6), 73.65 (C-3), 77.97 (C-28), 86.15 (C-7), 87.56 (C-15), 88.10 (C-1), 109.93 (C-22), 126.47 (C-20), 135.41 (C-13), 138.40 (C-21), 143.44 (C-23), 145.61 (C-14), 171.06 (C-12); *m/z* 440 (M^+), 407, 363, 244, 200.

1,2-Didehydro-1,3-dideoxy-3-oxosalannic acid ***18***. Mp 176–178 °C (from acetone); $[\alpha]_D^{25}$ +170 (*c* 1, $CHCl_3$), ν_{max}/cm^{-1} 3500 br, 1728, 1684; δ_H (300 MHz) 1.22 (3H, s, H_3-29), 1.30 (3H, s, H_3-19), 1.37 (3H, s, H_3-30), 1.74 (3H, d, *J* 1.5, H_3-18), 1.98–2.17 (1H, m, H-9), 2.09–2.25 (2H, m, H_2-16), 2.46–2.71 (2H, m, H_2-11), 2.66 (1H, d, *J* 12.5, H-5), 3.66 (1H, d, *J* 6.9, H-17), 3.89 (1H, d, *J* 8.0, H^a-28), 4.02 (1H, d, *J* 8, H^b-28), 4.09 (1H, dd, *J* 12.5 and 3.3, H-6), 4.22 (1H, d, *J* 3.3, H-7), 5.39 (1H, s, H-15), 5.78 (1H, d, *J* 10, H-2), 6.17 (1H, s, H-22), 6.76 (1H, d, *J* 10, H-1), 7.13 (1H, s, H-21), 7.26 (1H, s, H-23); δ_C (100 MHz) 13.14 (C-18), 15.42 (C-30), 17.64 (C-19), 18.35 (C-29), 31.32 (C-11), 40.06 (C-10), 41.62 (C-16), 45.09 (C-9), 48.94 (C-4), 49.51 (C-5), 49.62 (C-17), 49.88 (C-8), 72.49 (C-6), 77.06 (C-28), 86.31 (C-7), 87.97 (C-15), 110.29 (C-22), 126.79 (C-20), 127.54 (C-2), 135.63 (C-13), 138.66 (C-21), 143.12 (C-23), 145.92 (C-14), 154.52 (C-1), 177.61 (C-12), 201.02 (C-3); *m/z* 437 ($M^+ - 1$), 406, 392, 245 (EI-HRMS): Found: M^+, 438.204412. $C_{26}H_{30}O_6$ requires *M*, 438.204239.

3-Deoxy-3-oxosalannol ***20***. Mp 219–221 °C (from EtOAc); $[\alpha]_D^{25}$ +136 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 3412, 1713; δ_H (300 MHz) 1.11 (3H, s, H_3-30), 1.32 (3H, s, H_3-29), 1.38 (3H, s, H_3-19), 1.71 (3H, d, *J* 1.2, H_3-18), 2.05–2.27 (3H, m, H-9, H_2-16), 2.38 (1H, dd, *J* 15 and 2.7, H^a-2), 2.69 (1H, d, *J* 12.3, H-5), 3.08 (1H, dd, *J* 15 and 3.6, H^b-2), 3.47–3.77 (4H, m, H_2-12, H-17, H^a-28), 4.01 (1H, d, *J* 8, H^b-28), 4.06–4.16 (2H, m, H-1, -6), 4.20 (1H, d, *J* 3.3, H-7), 5.43–5.48 (1H, m, H-15), 6.19 (1H, s, H-22), 7.19 (1H, s, H-21), 7.34 (1H, t, *J* 1.6, H-23); δ_C (100 MHz) 12.84 (C-18), 15.15 (C-30), 16.94 (C-19), 18.32 (C-29), 27.74 (C-11), 38.19 (C-9), 41.11 (C-10), 41.18 (C-16), 42.79 (C-4), 45.01 (C-5), 49.22 (C-17), 49.51 (C-8), 52.69 (C-2), 62.17 (C-12), 72.59 (C-6), 75.92 (C-28), 77.09 (C-1), 85.63 (C-7), 87.68 (C-15), 110.01 (C-22), 126.97 (C-20), 133.53 (C-13), 138.35 (C-21), 143.35 (C-23), 147.22 (C-14), 210.27 (C-3); *m/z* 442 (M^+), 424 ($M^+ - H_2O$), 411, 399, 381 (EI-HRMS): Found: M^+, 442.2366. $C_{26}H_{34}O_6$ requires *M*, 442.2355.

1,2-Didehydro-1,3-dideoxy-3-oxosalannol ***23***. Mp 246–249 °C (from acetone): $[\alpha]_D^{25}$ +221 (*c* 1, $CHCl_3$); ν_{max}/cm^{-1} 3463, 1683 (Found: C, 73.96; H, 7.73. $C_{26}H_{32}O_5$ requires C, 73.58; H, 7.55%); δ_H (300 MHz) 1.19 (3H, s, H_3-30), 1.29 (3H, s, H_3-29), 1.37 (3H, s, H_3-19), 1.77 (3H, d, *J* 1.5, H-18), 1.63–2.28 (5H, m, H-9, H_2-11, -16), 2.68 (1H, d, *J* 12, H-5), 3.55–3.69 (3H, m, H_2-12, H-17), 3.88 (1H, d, *J* 8, H^a-28), 4.01 (1H, d, *J* 8, H^b-28), 4.14 (1H, dd, *J* 12 and 3, H-6), 4.24 (1H, d, *J* 3, H-7), 5.33–5.38 (1H, m, H-15), 5.86 (1H, d, *J* 10, H-2), 6.17 (1H, s, H-22), 6.98 (1H, d, *J* 10, H-1), 7.20 (1H, s, H-21), 7.34 (1H, t, *J* 1.7, H-23); δ_C (100 MHz) 13.27 (C-18), 15.44 (C-30), 17.66 (C-19), 18.33 (C-29), 29.32 (C-11), 40.11 (C-10), 41.30 (C-16), 44.13 (C-9), 48.93 (C-4), 49.40 (C-5), 49.46 (C-8), 49.56 (C-17), 62.17 (C-12), 72.53 (C-6), 76.97 (C-28), 86.36 (C-7), 87.30 (C-15), 109.83 (C-22), 127.00 (C-20), 127.12 (C-2), 134.40 (C-13), 138.41 (C-21), 143.34 (C-23), 146.74 (C-14), 155.86 (C-1), 201.36 (C-3); *m/z* 424 (M^+), 409 ($M^+ - CH_3$), 393, 231.

Acknowledgements

We thank Professor S. B. Sulia, Department of Microbiology, Bangalore University, for identifying the strain. Financial assistance from CSIR (New Delhi, India) is gratefully acknowledged. Financial support to K. V. by I.I.Sc is greatly appreciated.

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