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SALANNOLIDE, A MELIACIN FROM *AZADIRACHTA INDICA**

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Key Word Index—*Azadirachta indica*, Meliaceae, nim, seed oil bitters, tetra-nor-triterpenoid, salannolide

Abstract—The structure of a new meliacin named salannolide has been elucidated by physicochemical data The unique feature of this compound is the presence of a hydroxybutenolide side chain in place of the usual furan ring attached at C-17

INTRODUCTION

Recently, a number of tetra-nor-triterpenoids (meliacins) belonging to the azadirone and the nimbin/salannin types have been isolated from the seeds of *Azadirachta indica* A Juss (syn *Melia azadirachta*) commonly known as neem (nim) [1–3]

RESULTS AND DISCUSSION

The total bitter principles [4] isolated from fresh neem seed oil by column chromatography over silica gel (C_6H_6–EtOAc, 2 3) yielded a new meliacin (80 mg/kg seed oil) named salannolide We report here the structure of salannolide (**1**)

Salannolide (**1**), mp > 320° (dec), $[\alpha]_D^{27}$ +185° (*c* 1 0, $CHCl_3$) analysed for $C_{34}H_{44}O_{11}$ (M^+ *m/z* 628, FDMS) The IR spectrum of salannolide showed the presence of a hydroxyl group (3400 cm^{-1}) and multiple bands in the carbonyl region (1765 cm^{-1} unsaturated lactone carbonyl, 1720 cm^{-1} tiglate/acetate carbonyl and 1700 cm^{-1} ester carbonyl) and the presence of unsaturation (1650 and 820 cm^{-1} trisubstituted double bonds) The other characteristic feature of the IR spectrum was the presence of oxide/ether functions (1150 and 1080 cm^{-1}) Interestingly, the characteristic absorption around 880 cm^{-1} for a furan grouping, a usual feature of the meliacins, was missing in the IR spectrum of salannolide The absence of the furan ring was also evident from the ^1H NMR spectrum The mass spectrum of salannolide ($[M]^+$ at *m/z* 628) showed the loss of a water molecule from the parent ion to give a peak at *m/z* 610 indicating the presence of a hydroxyl group in the molecule The other significant fragmentation in the mass spectrum of **1** was the loss of the tiglic acid moiety both from the molecular ion to give a peak at *m/z* 528 $[M - C_5H_8O_2]^+$ and on successive loss of water at *m/z* 510 The base ion peak at *m/z* 83 corresponded to the tiglyl ion $[C_4H_7CO]^+$

The ^1H NMR spectrum of salannolide (**1**) showed the presence of three angular methyl groups at δ0 9, 1 1 and 1 2, respectively, together with a vinylic methyl at 1 67 and an ester methyl at 3 28 The methyls of the tiglate moiety appeared between 2 1 and 1 9 The comparison of the ^1H NMR spectra of salannolide (**1**) with those of salannin (**2**) [5] and nimbidinin triacetate (**3**) [6] revealed two interesting features Firstly, the identities of the ester functions of **1** as tiglate and acetate at C-1 and C-3, respectively, were indicated by the presence of two diffuse one-proton triplets at δ4 9 and 4 8 similar to those reported for salannin and nimbidinin triacetate (each proton at C-1 and C-3 being individually coupled to the methylene protons at C-2) Furthermore, the shielding of the ester methyl (δ3 25 in salannin and 3 28 in salannolide) indicated that the tiglate was at C-1 and the acetate was at C-3 as in salannin (**2**) [5] and that the ring C was *seco* with a carboxymethyl at C-12 Secondly, the C-5, C-6, C-7 carbon chain in salannolide resembled that of salannin in the following manner The H-6 proton, a double-doublet centred at δ3 9 was coupled with the H-5 proton (J = 10 Hz) and the H-7 proton (J = 2 5 Hz) as in salannin (**2**) [5] The H-7 proton in turn appeared at δ4 15 (*d*, J = 2 5 Hz) while H-5 appeared at 2 7 (*d*, J = 10 Hz) The protons at C-5 and C-7 are otherwise not coupled, thereby indicating the absence of protons at C-8 and C-10 and that the configuration of the protons at C-5, C-6 and C-7 is axial, axial and equatorial, respectively The chemical shifts of H-6 and H-7 are very similar to those reported for salannin The oxide links in salannolide are therefore through C-6 and C-28, and C-7 and C-15, respectively This was fully supported by a two-proton AB quartet at δ3 50 adjacent to an ether function assigned to the C-28 methylene, while H-15 appeared as a broad triplet at 5 3 showing coupling with the methylene at C-16 and long-range couplings with the vinylic methyl at C-13

The data given above thus clearly suggest that the basic skeleton of salannolide constituting rings A, B, C and D was identical to that reported for salannin in all respects and also accounted for eight of the eleven oxygens in the molecule The ^1H NMR spectrum of salannolide, however, differed from that of salannin in the olefinic region The protons α to the oxygen of the furan ring appearing at δ7 2–7 4 and the β proton at 6 3 in salannin were absent in salannolide and instead two protons appeared at 5 8 (J = 1 5 Hz) and 7 2 (J = 1 5 Hz) The olefinic proton of the tiglate moiety appeared at δ6 85 Salannolide (**1**) thus appeared to be different from salannin in respect of the nature of the furan ring attached at C-17 The ^{13}C NMR spectrum of salannolide on comparison with that of

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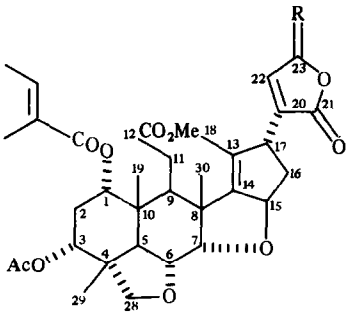

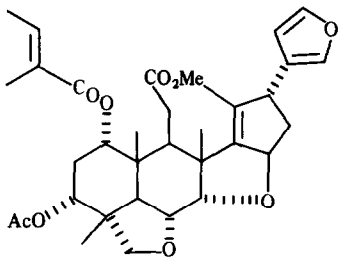

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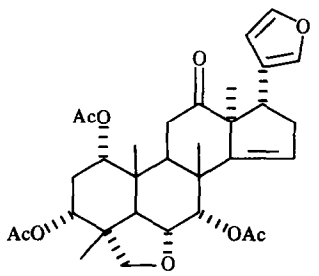
1 R = H, OH

1a R = H, OTMS

1b R = H, OAc



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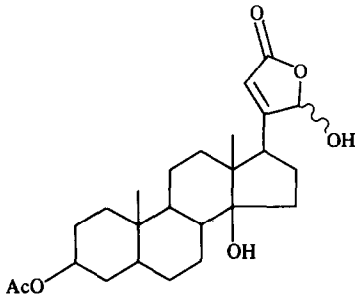

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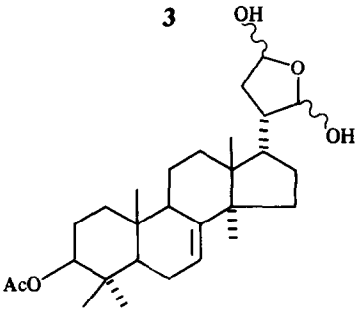

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salannin [1] (Table 1) revealed two interesting features Firstly, a doublet at δ96 88 indicated the presence of a hemiacetal type of carbon and secondly, an additional carbon resonance appeared at 170 7, the latter signal could be assigned to an α,β-unsaturated-γ-lactone The other two carbons of the side chain appeared as olefinic carbons at δ137 5 as a singlet (C-20) and at 142 01 as a doublet (C-22) The rest of the ^{13}C NMR spectrum of **1** was comparable to that reported [1] for salannin (Table 1) Thus salannolide [1] had a 23-hydroxy-20-(22)-butene-(21 → 23)γ-lactone grouping attached at C-17 in place of the normal furan ring The presence of the hydroxyl group in **1** was further supported by silylation *in situ* and recording the mass spectrum The silyl salannolide (**1a**) gave the molecular ion at *m/z* 700, which showed the loss of 90 mass units due to Me_3SiOH to give a peak at *m/z* 610 Acetylation of **1** at room temperature with Ac_2O–pyridine and work-up within 2 hr yielded the acetate **1b**, mp 204–206°, $C_{36}H_{46}O_{12}$ ([M]$^+$ at *m/z* 670) The ^{1}H NMR spectrum of **1b** showed the shift of the methine proton of the hemiacetal at δ5 8 in **1** to 6 8 in **1b**

The ^{1}H NMR spectrum of **1** in conjuction with the ^{13}C NMR spectrum suggested that a hydroxyl group was

Table 1 ^{13}C NMR spectral data of salannolide (**1**) and salannin (**2**) [1] (20 MHz, $CDCl_3$, TMS as internal standard)

C No	1	2	C No	1	2
1	72 54 *d*	72 56 *d*	18	15 33 *q*	15 08 *q*
2	28 14 *t*	27 53 *t*	19	16 86 *q*	16 86 *q*
3	70 75 *d*	71 32 *d*	20	137 50 *s*	120 00 *s*
4	42 73 *s*	42 70 *s*	21	170 70 *s*	138 70 *d*
5	40 25 *d*	39 93 *d*	22	142 01 *d*	110 54 *d*
6	71 29 *d*	71 32 *d*	23	96 88 *d*	142 80 *d*
7	86 05 *d*	85 65 *d*	28	77 69 *t*	77 60 *t*
8	48 46 *s*	49 06 *s*	29	19 10 *q*	19 57 *q*
9	39 25 *d*	39 43 *d*	30	13 28 *q*	13 00 *q*
10	40 63 *d*	40 59 *d*	MeCO	170 35 *s*	170 02 *s*
11	30 05 *t*	30 67 *t*	CH_3CO	20 95 *q*	20 01 *q*
12	174 46 *s*	172 70 *s*	$COOCH_3$	52 46 *q*	51 20 *q*
13	132 73 *s*	134 80 *s*	1′	166 56 *s*	166 22 *s*
14	148 03 *s*	146 40 *s*	2′	129 03 *s*	129 00 *s*
15	87 48 *d*	87 66 *d*	3′	137 32 *d*	137 10 *d*
16	41 18 *t*	41 41 *t*	4′	11 91 *q*	11 39 *q*
17	48 79 *d*	49 40 *d*	5′	14 36 *q*	14 34 *q*

present at C-23 rather than at C-21 as the olefinic proton at δ7 2 could be assigned to that at C-22 (β to the carbonyl) The chemical shifts [7] of the isomeric hydroxybutenolide (4) having a hydroxyl group at C-21 and a carbonyl group at C-23 synthesized during the preparation of cardenolides from furyl androstanes [7] are reported to be δ5 8 and 5 9 for the C-21 and C-22 protons, respectively This fully established the structure of salannolide as 1

This is the first report of the natural occurrence of a hydroxybutenolide side chain present in a meliacin and it is of biogenetic significance This compound could be related to the dihemiacetal (5), prepared [8] by degradation of turreanthin, as an intermediate in the possible route for the formation of the furan ring in meliacins

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ISOLATION AND STRUCTURES OF DIOMUSCINONE AND DIOMUSCIPULONE FROM *DIONAEA MUSCIPULA*

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Key Word Index—*Dionaea muscipula*, Droseraceae, phenolic compounds, diomuscinone, diomuscipulone

Abstract—From the fresh leaves and roots of *Dionaea muscipula*, two new substances (diomuscinone and diomuscipulone) have been isolated together with the known naphthoquinone plumbagin The structures of the new compounds have been elucidated on the basis of their spectral data coupled with some chemical evidence

INTRODUCTION

From *Dionaea muscipula* E, we have isolated two new interesting compounds named diomuscinone (1) and diomuscipulone (2), in addition to the known naphthoquinone, plumbagin (3) [1, 2] From a biogenetic point of view, the newly isolated substances (1 and 2) seem to be related to plumbagin (3), which is the principal component This paper describes the isolation and structures of diomuscinone and diomuscipulone Furthermore, the biogenetic relationship between diomuscipulone (2) and plumbagin (3) is also demonstrated

RESULTS AND DISCUSSION

The ethyl acetate soluble part of the methanol extract of *D muscipula* was separated by a combination of silica gel column chromatography and preparative TLC to afford diomuscinone (1), diomuscipulone (2) and plumbagin (3) [1, 2], in 0 024, 0 014 and 2 1% yields (from weight of the methanol extract), respectively

Diomuscinone (1), molecular formula $C_{12}H_{12}O_4$, has two CO groups (δ200 8 and 202 8) and a tri-substituted aromatic ring (δ118 7, 124 0 and 136 9) The presence of an Me–C(–)–CH_2OH grouping is suggested on the basis of its ^1H and ^{13}C NMR spectra [δ1 31 (3H, *s*), 3 53 (1H, *d*, J = 11 5 Hz) and 4 06 (1H, *d*, J = 11 5 Hz), δ21 4 (*q*), 50 5 (*s*) and 67 8 (*t*)] On the basis of these and other spectral data and the following chemical evidence together with co-occurrence of plumbagin (3) as a main component, the structure of diomuscinone must be represented by 1 When treated with 60% sodium hydride in mineral oil at room temperature for 12 hr, diomuscinone (1) is readily converted into plumbagin (3), in 82% yield, via the corresponding hydroquinone-type intermediate (4).

Diomuscipulone (2), with a molecular formula $C_{12}H_{12}O_5$, has one ketonic carbonyl group (1700 cm^{-1}), one hydroxyl group and a tri-substituted aromatic ring [δ6 85–7 25 (3H, complex)] Its ^1H NMR and mass spectra {δ1 43 (3H, *s*), 2 99 (2H, *s*) and 3 50 (3H, *s*), *m/z* 177 $[M-COOMe]^+$ and 163 $[M-CH_2COOMe]^+$} show that it contains partial structure A, which must be further extended to B on the basis of the following chemical