ARANOROSINOL A AND ARANOROSINOL B, TWO NEW METABOLITES FROM *Pseudoarachniotus roseus*: PRODUCTION, ISOLATION, STRUCTURE ELUCIDATION AND BIOLOGICAL PROPERTIES†

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(Received for publication April 20, 1992)

Two new secondary metabolites, aranorosinol A (**1**) and aranorosinol B (**2**), were isolated from a strain of *Pseudoarachniotus roseus*. Their structures were elucidated on the basis of their spectral properties and chemical transformations and were found to be similar to aranorosin (**3**) isolated from the same strain.

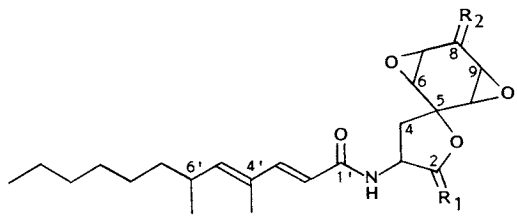
We have recently isolated two new metabolites, aranorosinol A (**1**) and aranorosinol B (**2**), from the fungal strain Y-30499 identified as *Pseudoarachniotus roseus*. Herein we report the isolation, structure elucidation and biological properties of these two metabolites. Compounds **1** and **2** are structurally related to aranorosin (**3**) which was isolated earlier from the same fungal strain[1~3].

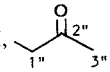
Fermentation

Fermentation was carried out in shake flasks as well as in laboratory fermenters using the methods and conditions reported earlier[1]. The progress of the fermentation was monitored by the agar diffusion assay using *Bacillus subtilis* as the test strain. The antibiotics were present in both the culture filtrate and the mycelial cake.

Isolation

The culture filtrate (40 liters) was extracted with ethyl acetate (2 × 15 liters). The mycelial cake (3.7 kg)



1 $R_1=R_2=H$, OH
2 $R_1=H$, OH; $R_2=OH$, 
3 $R_1=H$, OH; $R_2=O$
4 $R_1=R_2=H$, $OCOCH_3$
5 $R_1=R_2=O$
6 $R_1=H$, $OCOCH_3$; $R_2=OH$, CH_2COCH_3
7 $R_1=H$, $OCOCH_3$; $R_2=OCOCH_3$, CH_2COCH_3

† Dedicated to Prof. Ch. BHEEMASANKARA RAO of Andhra University on the occasion of his 60th birthday.

Table 1. Physico-chemical characteristics of aranorosinol A (**1**) and aranorosinol B (**2**).

	Aranorosinol A	Aranorosinol B
Nature	White powder	White powder
MP	133~135°C	84~85°C
Solubility	CH_2Cl_2, $CHCl_3$, EtOAc, acetone and MeOH	CH_2Cl_2, $CHCl_3$, EtOAc, acetone and MeOH
$[\alpha]_D$	−25.06° (*c* 7.82, MeOH)	−9.3° (*c* 0.4, $CHCl_3$)
TLC Rf	0.38[a], 0.13[b]	0.55[a], 0.25[b]
FAB-MS (glycerol matrix)	422 $(M+H)^+$, 428 $(M+Li)^+$, 444 $(M+Na)^+$	478 $(M+H)^+$
Elemental Analysis	$C_{23}H_{35}NO_6 \cdot H_2O$	$C_{26}H_{39}NO_7$
Found:	C 62.45, H 8.32, N 3.45	C 64.91, H 8.89, N 3.27
Calcd:	C 62.87, H 8.43, N 3.19	C 65.40, H 8.18, N 2.93
UV λ_{max} (MeOH) nm	266	265
IR (KBr) cm^{-1}	3400, 1640, 1600, 1525, 1440, 1250, 1075, 980, 935, 845, 770 and 725	3380, 1720, 1670, 1630, 1550, 1460, 1280, 1040, 1000, 935, 900, 860 and 745

[a] $CHCl_3$-MeOH (85:15).
[b] Ethyl acetate.

was extracted with acetone (2×8 liters). The combined acetone extracts were concentrated under reduced pressure, diluted with water and extracted with ethyl acetate (2×750 ml). These ethyl acetate extracts of mycelial cake were combined with those of the culture filtrate and concentrated under reduced pressure to give 18.7 g of the crude antibiotic mixture as a reddish brown syrupy material. This was flash chromatographed on silica gel (230~400 mesh, 250 g, flow rate: 50 ml/minute) using $CHCl_3$ and a gradient of MeOH in steps of 1% for elution. A mixture of aranorosinol B (**2**) and aranorosin (**3**) was eluted with 2~3% MeOH in $CHCl_3$, while aranorosinol A (**1**) was eluted with 5% MeOH in $CHCl_3$.

The semipure aranorosinol A (**1**) (3.7 g) was flash chromatographed on silica gel (230~400 mesh, 60 g) with a flow rate of 40 ml/minute using $CHCl_3$-MeOH (95:5) as the eluent. The active eluates were evaporated to dryness under reduced pressure, dissolved in $CHCl_3$ and pure aranorosinol A (**1**) was precipitated as a white solid (1.2 g) by adding hexane. The mixture (3.6 g) of aranorosinol B (**2**) and aranorosin (**3**) was separated on a silica gel (230~400 mesh, 150 g) column using petroleum ether (60~70°C)-ethyl acetate mixtures for elution. Pure aranorosin (**3**) was eluted with petroleum ether-ethyl acetate (1:1), while aranorosinol B (**2**) was eluted with petroleum ether-ethyl acetate (1:3). The eluates containing **2** were evaporated to dryness, dissolved in $CHCl_3$ and precipitated with hexane to give pure aranorosinol B as a white solid (40 mg).

The physico-chemical characteristics of aranorosinol A (**1**) and aranorosinol B (**2**) are listed in Table 1.

Structure Elucidation

The ^1H and ^{13}C NMR data of aranorosinol A (**1**) and aranorosinol B (**2**) are listed in Table 2. A comparison of the ^1H and ^{13}C NMR spectra of aranorosinol A (**1**) and aranorosinol B (**2**) with those of aranorosin (**3**)[2,3] showed close structural similarity of the compounds; all three compounds had the same fatty acid side chain (4,6-dimethyldodeca-2*E*,4*E*-dienoyl moiety) and the same 1-oxaspiro[4.5]decane ring system. Structural differences between **1**, **2** and **3** were observed at the C-8 position only.

Aranorosinol A (1)

Aranorosinol A (**1**), $C_{23}H_{35}NO_6$, differs from **3** in having two additional hydrogens in the molecule.

Table 2. ^{13}C and ^{1}H NMR data of aranorosinol A (**1**), aranorosinol B (**2**) and aranorosin (**3**).

Position	^{13}C (δ)[a]			^{1}H (δ, Multiplicities, coupling constants in Hz)[b]		
	1[c]	**2**[d]	**3**[e]	**1**[c]	**2**[c]	**3**[c]
2	96.55	96.65	96.60	5.54 (d, 4.4)	5.56 (d, 4.5)	5.63 (d, 4.3)
3	52.73	52.44	52.00	4.68 (m)	4.72 (m)	4.80 (m)
4	37.35	37.05	35.90	2.48 (m, H_a);	2.60 (dd, 8.5, 12.8, H_a);	2.65 (dd, 8.6, 13.0, H_a);
				1.98 (dd, 10.8, 12.6, H_b)	2.01 (dd, 10.8, 12.8, H_b)	2.06 (dd, 10.6, 13.0, H_b)
5	80.51	78.99	78.80	—	—	—
6	61.41	59.59	64.40	3.44 (m)	3.31 (dd, 3.4, 3.7)	3.68 (dd, 3.9, 3.5)
7	57.18	58.19	55.80	3.44 (m)	3.25 (m)	3.44 (dd, 3.5, 2.9)
8	64.15	66.42	198.40	4.28 (t, 2.7)	—	—
9	56.96	57.54	55.60	3.44 (m)	3.25 (m)	3.46 (dd, 3.5, 2.9)
10	60.07	58.73	63.00	3.44 (m)	3.25 (dd, 3.4, 2.5)	3.57 (dd, 3.9, 3.5)
1′	167.31	166.87	167.00	—	—	—
2′	118.01	117.89	117.00	5.85 (d, 15.4)	5.78 (d, 15.4)	5.77 (d, 15.3)
3′	146.72	146.94	147.40	7.25 (d, 15.4)	7.24 (d, 15.4)	7.25 (d, 15.3)
4′	131.23	131.11	130.80	—	—	—
5′	147.69	147.69	148.50	5.68 (d, 9.3)	5.66 (d, 9.7)	5.67 (broad d)
6′	33.26	33.26	33.20	2.45 (m)	2.49 (m)	2.52 (m)
7′	37.38	37.38	37.20	1.26 (broad s)	1.23 (broad s)	1.27 (broad s)
8′	27.52	27.63	27.40	1.26 (broad s)	1.23 (broad s)	1.27 (broad s)
9′	29.47	29.58	29.40	1.26 (broad s)	1.23 (broad s)	1.27 (broad s)
10′	31.85	31.96	31.80	1.26 (broad s)	1.23 (broad s)	1.27 (broad s)
11′	22.75	22.75	22.60	1.26 (broad s)	1.23 (broad s)	1.27 (broad s)
4′-CH_3	12.56	12.57	12.40	1.79 (d, 0.9)	1.76 (d, 1.0)	1.78 (d, 0.3)
6′-CH_3	20.58	20.48	20.50	0.99 (d, 6.7)	0.97 (d, 6.7)	0.98 (d, 6.7)
11′-CH_3	14.08	14.08	14.10	0.89 (t, 6.5)	0.87 (t, 6.5)	0.88 (t, 6.8)
2-OH	—	—	—	6.74 (broad s)[f]	5.54 (broad s)	4.26 (broad s)
8-OH	—	—	—	5.43 (broad s)[f]	4.69 (broad s)	—
NH	—	—	—	6.63 (d, 8.0)	6.20 (d, 8.3)	6.09 (d, 8.2)
1″	—	47.45	—	—	3.07 (s)	—
2″	—	210.44	—	—	—	—
3″	—	31.53	—	—	2.28 (s)	—

[a] Spectra were taken at 22.5 MHz using 50 mg/ml concentrations and the assignments of **1** and **2** were based on comparison with those reported for aranorosin (**3**).
[b] Spectra were taken at 300 MHz using 10 mg/ml concentrations.
[c] $CDCl_3$.
[d] $CDCl_3$-C_6D_6 (3:1).
[e] $CDCl_3$-CD_3OD (8:2).
[f] These chemical shifts were obtained from ^{1}H NMR spectrum taken in DMSO-d_6.

The ^{1}H NMR spectrum shows three D_2O exchangeable protons (δ 6.74, 6.63 and 5.43) as compared to the presence of two D_2O exchangeable protons in **3**. This observation gave an early indication that **1** might be a reduced form of **3**.

The ^{13}C NMR data of **1** showed the absence of the C-8 carbonyl group of **3** and the presence of a new signal at δ 64.15 (–CH(O)/DEPT-135). In the ^{1}H NMR spectrum, this oxymethine proton appeared at δ 4.28 (t, J=2.7 Hz). Acetylation of **1** with acetic anhydride-pyridine gave a diacetate (**4**) ($C_{27}H_{39}NO_8$, MP 184~186°C, EI-MS: m/z 505 ($M^{+\cdot}$)) in which the H-8 proton underwent a downfield shift of about 1δ to δ 5.36 (t, J=3 Hz), as expected on acetylation of a secondary hydroxyl group. The other acetylated group in **4** was the hemiacetal group in which the H-2 proton appeared at δ 6.41, a downfield

shift of 0.9 δ from its original position at δ 5.54 in **1**. All these data indicated that aranorosinol A (**1**), instead of the C-8 carbonyl group present in **3**, contained an 8-CH(OH) moiety. This was further confirmed by chemical conversion of **3** to **1**. Thus, $NaBH_4$ reduction of aranorosin (**3**) yielded a diastereoisomerically pure C-8 alcohol which was identical by ^1H NMR (300 MHz, $CDCl_3$ - CD_3OD) to the naturally occurring alcohol aranorosinol A (**1**). Furthermore, Jones oxidation[4] of **1** gave a product which was identical (TLC, MP, IR, ^1H and ^{13}C NMR spectra) to the ketolactone (**5**)[2] obtained by Jones oxidation of aranorosin (**3**). A chemical shift correlated 2D ^1H-^1H COSY spectrum of **1** recorded in DMSO-d_6 - D_2O mixture further confirmed the proton connectivities and the structure of aranorosinol A was thus established to be **1**.

Stereochemistry of **1**: The direct chemical conversion of **3** to **1** established the stereochemistry of all the protons in **1** except the H-8 proton. 2D NOESY as well as difference NOE data showed the expected cross-peaks, thus, confirming the stereochemistry of the diene system and also the epoxy protons. Furthermore, NOESY cross-peaks were observed between the H-8 proton and the epoxy protons indicating their proximal orientation, but the exact relative configuration at C-8 remains uncertain.

Aranorosinol B (**2**)

As in the case of **1**, aranorosinol B (**2**), $C_{26}H_{39}NO_7$, had three D_2O exchangeable protons (δ 6.20, 5.54 and 4.69), an oxygenated carbon signal at δ 66.42 in the ^{13}C NMR spectrum and lacked the C-8 carbonyl group of **3**. In the ^{13}C NMR spectrum of **2**, three additional carbons appeared at δ 210.44 (carbonyl group), δ 47.45 (CH_2/DEPT-135) and δ 31.53 (CH_3/DEPT-135). The CH_2 and CH_3 groups were isolated spin systems and appeared as singlets in the ^1H NMR spectrum at δ 3.07 and δ 2.28, respectively. The above data are best explained by an 8-$C(OH)CH_2COCH_3$ moiety. The presence of such a group is known from the enaminomycins[5].

Aranorosinol B (**2**) underwent monoacetylation with acetic anhydride - pyridine (1.5 equiv, 24 hours at ambient temperature) to give **6**, $C_{28}H_{41}NO_8$. It was characterised by the IR spectrum (1755 cm^{-1}, *O*-acetyl) and the deshielding of the H-2 proton from δ 5.56 to 6.41 in the ^1H NMR spectrum. However, with excess of acetic anhydride - pyridine (5 equiv, 24 hours at ambient temperature) a mixture of **6** and a diacetate (**7**), in which the 8-hydroxyl group was also acetylated, was formed. These could be separated on a silica gel column (230~400 mesh) using $CHCl_3$ - MeOH mixtures for elution.

The proposed structure of **2** had a β-hydroxycarbonyl group which was appropriate for a retro-aldol condensation to give **3**. Reaction of **2** with butylamine (20 equiv) in DMSO (3 hours at ambient temperature) indeed gave a product which was identical to **3** by MP, TLC, IR, ^1H and ^{13}C NMR, thus establishing the structural relationships between **2** and **3**. A 2D ^1H-^1H COSY spectrum of **2** further confirmed the proton connectivities and the structure of aranorosinol B was thus established to be **2**.

Table 3. MIC values of aranorosinol A (**1**) and aranorosinol B (**2**).

Test organisms	Minimum inhibitory concentration (μg/ml)	
	Aranorosinol A	Aranorosinol B
Staphylococcus aureus 209 P	15.6	31.25
S. aureus 20240	31.25	15.62
Bacillus subtilis	3.9	15.62
Streptococcus faecalis	31.25	125.00
Escherichia coli Ess 2231	62.5	>250.00
E. coli 9632	>250.00	>250.00
Proteus vulgaris	>250.00	>250.00
Salmonella typhimurium	>250.00	>250.00
Klebsiella pneumoniae	>250.00	>250.00
Pseudomonas aeruginosa	>250.00	>250.00
Candida albicans	62.5	250.00
Saccharomyces cerevisiae	31.25	250.00
Aspergillus niger	>250.00	>250.00
Trichophyton mentagrophytes	62.5	>250.00
Microsporum gypseum	>250.00	>250.00

Stereochemistry of **2**: Chemical conversion of **2** to **3** established the spatial relationships of the various protons in **2** to be the same as in **3** except at C-8. 2D NOESY and difference NOE experiments further supported this. NOESY cross-peaks were observed between the methylene protons H-1″ and the epoxy protons H-7 and H-9 indicating that the 8-CH_2COCH_3 group should be oriented close to the epoxy protons in the most favoured conformer of **2**. However, the relative stereochemistry of the groups at C-8 remains uncertain.

Biological Properties

The minimum inhibitory concentrations (MIC) of aranorosinol A (**1**) and aranorosinol B (**2**) required to inhibit a variety of bacterial and fungal strains are listed in Table 3. Both **1** and **2** possess only weak antibacterial and antifungal properties.

Experimental

UV spectra were recorded on a UVIKON 810 double beam spectrophotometer. IR spectra were taken on a Perkin-Elmer 157 spectrophotometer. Mass spectra were recorded on a Kratos MS 9025 spectrometer. Optical rotations were measured using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on Jeol FX90Q, Varian VXR 300 and Bruker ACP 300 instruments. DEPT, COSY and NOESY spectra were recorded using standard parameters. A mixing time of 500 milliseconds and 10 mmol solutions of **1** and **2** in $CDCl_3$-CD_3OD and $CDCl_3$ respectively were used for recording the NOESY spectrum. TLCs were carried out using pre-coated silica gel plates from E. Merck (for preparative TLC, Article No. 13794; for analytical TLC, Article No. 5554).

Aranorosinol A Diacetate (**4**)

To aranorosinol A (**1**) (50 mg, 0.12 mmol), acetic anhydride (0.1 ml, 1.06 mmol) and pyridine (0.1 ml, 1.24 mmol) were added and the mixture kept at room temperature for 16 hours. The crude diacetate (50 mg), obtained on usual work up, was purified by silica gel column chromatography (230~400 mesh, 5 g) using $CHCl_3$-MeOH mixtures for elution. The semi-pure diacetate was eluted with 1% MeOH in $CHCl_3$ and was further purified by preparative TLC using $CHCl_3$-MeOH (95:5) for developing the plates. Pure aranorosinol A diacetate (**4**) was obtained as a white powder from $CHCl_3$-hexane mixture. Yield 40 mg; MP 184~186°C; EI-MS *m/z* 505 ($M^{+}\cdot$); UV λ_{max} (MeOH) nm 266; IR (KBr) cm^{-1} 3333, 2924, 1730, 1718, 1639, 1603, 1515, 1361, 1227, 1111, 1031, 961, 919, 885 and 778; ^1H NMR (90 MHz, $CDCl_3$, TMS) δ 0.94 (t, J=6 Hz, CH_3), 0.98 (d, J=6 Hz, CH_3), 1.24 (broad s, 5×CH_2), 1.76 (s, CH_3), 2.00 (m, 2H), 2.16 (s, $OCOCH_3$), 2.20 (s, $OCOCH_3$), 2.56 (m, 1H), 3.23 (m, 2H), 3.38 (m, 2H), 4.92 (m, 1H), 5.36 (t, J=3 Hz, 1H), 5.62 (broad d, 1H), 5.66 (d, J=8 Hz, D_2O exchangeable, NH), 5.68 (d, J=15 Hz, 1H), 6.41 (d, J=5 Hz, 1H) and 7.22 (d, J=15 Hz, 1H). ^{13}C NMR (22.5 MHz, $CDCl_3$, TMS) δ 170.67 (s), 169.48 (s), 166.55 (s), 148.46 (d), 147.59 (d), 130.90 (s), 116.92 (d), 95.79 (d), 80.51 (s), 66.75 (d), 59.27 (d), 57.97 (d), 53.53 (d), 51.36 (d), 37.28 (t). 36.08 (t), 33.27 (d), 31.86 (t), 29.47 (t), 27.52 (t), 22.65 (t), 21.35 (q), 20.91 (q), 20.48 (q), 14.09 (q) and 12.57 (q).

Oxidation of Aranorosinol A (**1**) to the Ketolactone (**5**)

To aranorosinol A (**1**) (300 mg, 0.71 mmol) in acetone (10 ml), Jones reagent[4] (14 ml) was added slowly at room temperature over a period of 1 hour till the colour of the reagent persisted. The reaction mixture was then poured into ice water (50 ml) and extracted with CH_2Cl_2 (3×25 ml). The combined CH_2Cl_2 extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The crude product (138 mg) was purified by preparative TLC using MeOH-CH_2Cl_2 (10:90) for developing the plates. The pure product (95 mg) was identical to the ketolactone (**5**) derived from the oxidation of aranorosin (**3**)[2] by TLC, MP, IR, ^1H and ^{13}C NMR spectra.

Reduction of Aranorosin (**3**) to Aranorosinol A (**1**)

To aranorosin (**3**) (100 mg, 0.24 mmol) in THF (5 ml), sodium borohydride (9.4 mg, 0.25 mmol) was

added slowly at −20°C and the mixture was stirred at −20°C for 1 hour. The THF was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 ml), washed with water (3 × 15 ml) and the solvent removed under vacuum. The crude product (75 mg) was purified by preparative TLC using MeOH - CH_2Cl_2 (15 : 85) for developing the plates. The pure product (40 mg) was identical to aranorosinol A (**1**) by TLC, MP, IR, ^1H and ^{13}C NMR spectra.

Aranorosinol B Monoacetate (**6**)

To aranorosinol B (**2**) (48 mg, 0.1 mmol) in 2 ml of CH_2Cl_2, acetic anhydride (15 μl, 0.15 mmol) and pyridine (15 μl, 0.18 mmol) were added and the mixture kept at room temperature for 24 hours. The crude product (45 mg), obtained on usual work up, was purified by preparative TLC using $CHCl_3$ - MeOH (95 : 5) for developing the plates. The pure aranorosinol B monoacetate (**6**) was obtained as a white solid from $CHCl_3$ - hexane mixture. Yield 35 mg; MP 83 ~ 85°C; TLC Rf 0.43 (solvent $CHCl_3$ - MeOH (95 : 5)); CI-MS *m/z* 520 $(M+H)^+$; molecular formula $C_{28}H_{41}NO_8$; UV λ_{max} (MeOH) nm 264; IR (KBr) cm^{-1} 3500, 3380, 3280, 2985, 2940, 2860, 1755, 1715, 1660, 1620, 1545, 1380, 1240, 1075 and 1015. ^1H NMR (90 MHz, $CDCl_3$, TMS) δ 0.98 (t, J=6.4 Hz, CH_3), 1.01 (d, J=5.4 Hz, CH_3), 1.28 (broad s, 5 × CH_2), 1.80 (s, CH_3), 2.14 (m, 1H), 2.20 (s, CH_3), 2.32 (s, $OCOCH_3$), 2.50 (m, 1H), 2.62 (m, 1H), 3.08 (s, 2H), 3.24 (m, 4H), 4.85 (m, 1H), 5.60 (d, J=7.2 Hz, D_2O exchangeable), 5.65 (d, J=9 Hz, 1H), 5.68 (d, J=15 Hz, 1H), 6.41 (d, J=4.5 Hz) and 7.21 (d, J=15 Hz, 1H). ^{13}C NMR (22.5 MHz, $CDCl_3$, TMS) δ 210.65 (s), 169.26 (s), 166.54 (s), 148.45 (d), 148.12 (d), 130.90 (s), 116.92 (d), 95.90 (d), 80.51 (s), 66.42 (s), 58.62 (d), 58.29 (d), 57.97 (d), 56.99 (d), 51.36 (d), 47.35 (t), 37.27 (t), 36.62 (t), 33.26 (d), 31.85 (2 × q), 29.47 (t), 27.52 (t), 22.64 (t), 21.34 (t), 20.48 (q), 14.08 (q) and 12.56 (q).

Aranorosinol B Diacetate (**7**)

To aranorosinol B (**2**) (150 mg, 0.31 mmol), pyridine (0.2 ml, 2.47 mmol) and acetic anhydride (0.3 ml, 3.18 mmol) were added and the mixture kept at room temperature for 24 hours. The crude product, obtained on usual work up, was a mixture of mono- and di-acetates which was separated by silica gel column chromatography (230 ~ 400 mesh) using $CHCl_3$ - MeOH mixtures for elution.

The diacetate (**7**), eluted with 0.5% MeOH in $CHCl_3$, was obtained as a white solid from $CHCl_3$ - hexane mixture. Yield 40 mg; MP 85 ~ 88°C; TLC Rf 0.6 (solvent $CHCl_3$ - MeOH (95 : 5)); molecular formula $C_{30}H_{43}NO_9$; CI-MS *m/z* 562 $(M+H)^+$; UV λ_{max} (MeOH) nm 264; IR (KBr) cm^{-1} 3500, 3400, 3280, 2960, 2860, 1750, 1655, 1620, 1540, 1380, 1245, 1070, 1018 and 860. ^1H NMR (90 MHz, $CDCl_3$, TMS) δ 0.97 (t, J=6.4 Hz, CH_3), 1.01 (d, J=5.4 Hz, CH_3), 1.29 (broad s, 5 × CH_2), 1.80 (broad s, CH_3), 2.04 (m, 1H), 2.12 (s, $OCOCH_3$), 2.17 (s, CH_3), 2.27 (s, $OCOCH_3$), 2.48 (m, 1H), 2.88 (m, 1H), 3.20 (s, 2H), 3.22 (m, 2H), 3.46 (m, 2H), 4.90 (m, 1H), 5.59 (d, J=7 Hz, D_2O exchangeable, NH), 5.64 (d, J=9 Hz, 1H), 5.68 (d, J=15 Hz, 1H), 6.42 (d, J=4.5 Hz, 1H) and 7.22 (d, J=15 Hz, 1H). ^{13}C NMR (22.5 MHz, $CDCl_3$, TMS) δ 203.61 (s), 169.79 (s), 168.93 (s), 166.33 (s), 148.34 (d), 147.58 (d), 130.79 (s), 116.70 (d), 95.57 (d), 80.51 (s), 59.70 (s), 57.32 (4 × d), 51.25 (d), 46.92 (t), 37.81 (t), 37.27 (t), 33.26 (d), 31.85 (q), 31.42 (q), 29.36 (t), 27.41 (t), 22.64 (t), 21.34 (t), 20.48 (q), 13.97 (q) and 12.46 (q).

The pure monoacetate (65 mg), eluted with 1% MeOH in $CHCl_3$, was identical to **6** by TLC and ^1H NMR spectrum.

Conversion of Aranorosinol B (**2**) to Aranorosin (**3**)

To aranorosinol B (**2**) (500 mg, 1.05 mmol) in dry DMSO (10 ml), dry butylamine (2 ml, 20.2 mmol) was added dropwise with stirring. The resultant pale yellow solution was stirred at room temperature for 38 hours. The crude product (270 mg), obtained on usual work up, was chromatographed on silica gel (230 ~ 400 mesh) using $CHCl_3$ - MeOH mixtures for elution. The semi-pure product was further purified by preparative TLC using ethyl acetate for developing the plates. The pure product was identical to aranorosin (**3**) by TLC, UV, IR, ^1H and ^{13}C NMR and CI-MS spectra.

Acknowledgments

We thank Dr. P. K. INAMDAR of Hoechst India Limited for elemental analyses and recording some of the spectra. We thank Dr. H. W. FEHLHABER of Hoechst AG for his valuable comments and for recording some of the spectra. We

gratefully acknowledge the 300 MHz NMR facility of the Regional Sophisticated Instrumentation Centre (R.S.I.C.), Bombay, India. The technical assistance of Ms. BERNICE SMITH is acknowledged.

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