ARANOROSIN, A NOVEL ANTIBIOTIC FROM *PSEUDOARACHNIOTUS ROSEUS*

II. STRUCTURE ELUCIDATION†

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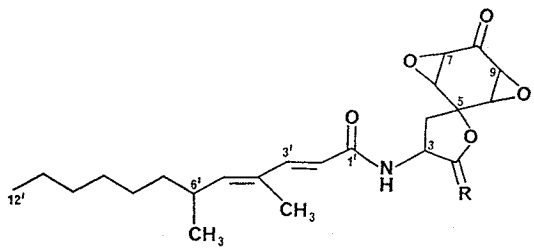
Aranorosin, a new antifungal antibiotic, has been isolated from the culture filtrate and mycelium of a strain of *Pseudoarachniotus roseus* Kuehn. The antibiotic, $C_{23}H_{33}NO_6$, contains a novel 1-oxaspiro[4,5]decane ring system. The structure (I) has been elucidated on the basis of spectroscopic and chemical analysis.

In the previous paper[1] the taxonomy and fermentation of the producing strain and the isolation, physico-chemical and biological properties of aranorosin (I) have been reported. We now describe the structure elucidation of the antibiotic based on extensive spectroscopic studies, especially with the aid of NMR correlation spectroscopy, as well as on chemical transformation reactions.

The high resolution mass spectrum (HR-MS) of aranorosin established its molecular formula as $C_{23}H_{33}NO_6$. The ^{13}C NMR spectrum showed that all of the 23 carbon atoms were magnetically non-equivalent and that two of these were carbonyl carbons, four olefinic carbons, seven heteroatom attached carbons and the remaining ten methine, methylene and methyl carbons. One of the two carbonyl groups had to be assigned to a keto function (198.4 ppm) and the other one, in accordance with an IR absorption at 1650 cm^{-1}, to an amide carbonyl (167.0 ppm).

The ^1H NMR spectrum showed the presence of a *trans*-olefin conjugated with a carbonyl function (doublets at 7.25 and 5.77 ppm, J=15.3 Hz) and a trisubstituted double bond linked to a methine group (doublet at 5.67 ppm, J=9.4 Hz). Considering the UV absorption at 264 nm, both double bonds should form a conjugated diene-one system. The ^1H NMR spectrum also showed a ten proton methylene signal and three different methyl signals (1.78, 0.98 and 0.88 ppm) indicating the presence of methyl groups attached to an olefin, a methine and a methylene group.

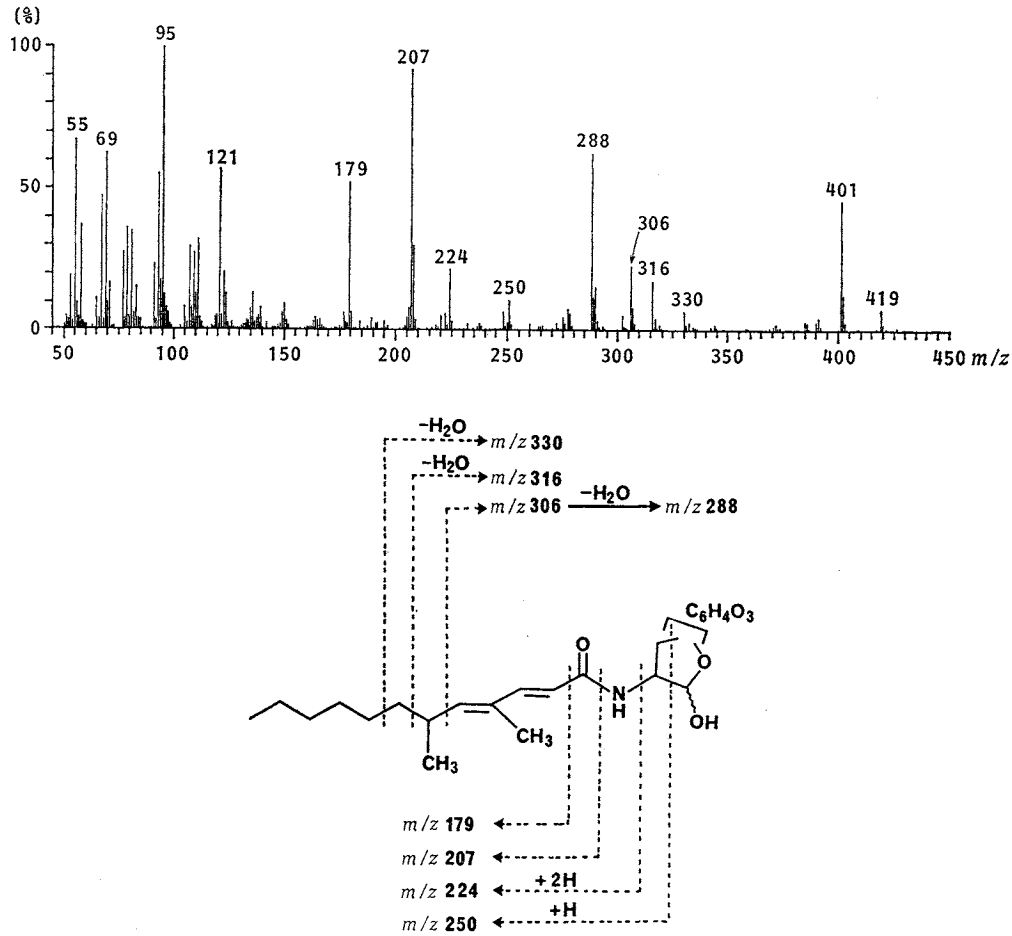


I R=H, OH
II R=H, $OCOCH_3$
III R=O

Further the ^1H NMR spectrum of aranorosin showed four mutually coupled protons (5.63,

† Dedicated to Prof. Dr. W. BARTMANN of Hoechst AG on the occasion of his 60th birthday.

Fig. 1. Mass spectrum and fragmentation pattern of aranorosin (I).

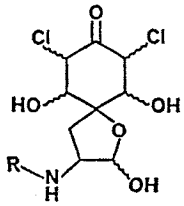


$M^{+\cdot}=m/z$ 419, $M-H_2O=m/z$ 401

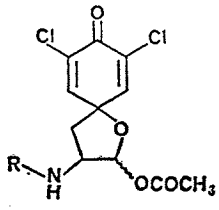
4.80, 2.65 and 2.06 ppm) and two D_2O exchangeable protons, only one of which could be acetylated (to **II**) or oxidized (to **III**). A thorough analysis of the splitting patterns of these signals led to the sequence $OCH(OH)CH(NHCO)CH_2C$. All these signals exhibited small satellite signals of about 25% intensity which became significantly smaller after treatment of the chloroform solution with D_2O thus indicating a slow equilibration between two epimeric acetal forms. Four additional protons appearing in the 3.4~3.7 ppm range which were coupled exclusively with each other could not be assigned at this stage of the structure elucidation.

Further information could be obtained from the electron impact (EI) induced degradation reactions. Using HR-MS, all significant fragment ions of aranorosin could be unequivocally assigned leading to the partial structure given in Fig. 1.

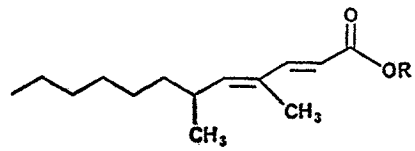
While the molecular formula of aranorosin suggested 8 double bond equivalents, the functional groups and partial structures established so far accounted for only 4. Since the remaining unsaturations are due to cyclic structures, aranorosin contains a tetracyclic ring system. Oxidation of the hemiacetal function by using either pyridinium dichromate in methylene chloride[2] or JONES reagent[3] led to the lactone **III**. Its IR spectrum displayed an additional carbonyl band at 1786 cm^{-1} indicating



IV



V



VI R=H
VII R=CH_3

the presence of a five membered lactone ring[4,5]. Hence aranorosin should also have a five membered ring incorporating the hemiacetal group.

The so far unrevealed part of the aranorosin structure, the $C_6H_4O_3$ moiety in the formula presented in Fig. 1, must contain the keto group, as known from the spectroscopic data, and two ether or epoxide oxygens. In order to elucidate this part of the molecule, an epoxide cleavage reaction using pyridinium chloride[6] was performed. The reaction product **IV** proved to be too labile to allow extensive spectroscopic investigations, but by dehydration with acetic anhydride - pyridine, the stable derivative **V** could be obtained. In the NMR spectrum of **V**, a pair of doublets appeared at 6.89 and 7.06 ppm (J=2.5 Hz) which is in good agreement with the calculated values obtained for the olefinic hydrogens of a 2,6-dichloro-2,5-cyclohexadiene-1-one system, but cannot exclude alternative structures unambiguously.

Attempts to cleave the amide bond and to isolate the amino compound containing the spiro system failed. However, after a reductive acetylation of aranorosin using zinc - acetic anhydride - sodium acetate, only the carboxylic acid **VI** could be isolated which was characterized by its methyl ester derivative **VII**.

NMR Correlation Spectroscopy

In order to achieve an unambiguous structure elucidation of aranorosin, a total analysis of its NMR spectra was performed using all measurable H-H, H-C and C-C spin-spin couplings. These can be detected most conveniently with the aid of appropriate two-dimensional (2D) NMR spectra[7~9]. As mentioned above the NMR spectra of aranorosin exhibit double sets of signals for many protons and carbons which arise from slow ring-opening and ring-closing reactions of the hemiacetal group resulting in a mixture of both C-2 epimers with an estimated ratio of 3 : 1. All NMR data and analysis presented here apply to the major isomer.

^{1}H-^{1}H correlation spectra (COSY)[7,8] at two different sample concentrations allowed the unequivocal assignment of all ^{1}H NMR signals together with their couplings (Table 1), and a ^{13}C-^{1}H correlation spectrum[10,11] led to the assignment of the ^{13}C NMR signals (Table 2). These data confirmed the substructures discussed previously. Furthermore, from the one bond C-H couplings obtained from a fully coupled ^{13}C spectrum, the quaternary carbon atoms C-5 and the two epoxide groups could be identified. The four epoxide carbons (C-6, C-7, C-9 and C-10) exhibit C, H couplings of 183~184 Hz which are typical for epoxides and clearly outside the range of other saturated alcohol or ether groups[12].

To clarify the connection of the various groups with the aid of long-range C-H couplings[13,14], several experiments optimized for different coupling constants were performed, but the results obtained (Table 2) did not lead to unambiguous evidence. So finally a ^{13}C-^{13}C correlation spectrum[15,16] was

Table 1. ^1H-^1H NMR correlation spectroscopic data of aranorosin in $CDCl_3$.

Proton	Chemical shift[a] (ppm), multiplicity	Coupling partner (coupling constant in Hz)[b]
3′-H	7.25 d	2′-H (15.3), 5′-H (0.3)
NH[c]	6.09, 6.35 d	3-H (8.2)
2′-H	5.77 d	3′-H (15.3), 5′-H (0.3)
5′-H	5.67 br d	6′-H (9.4), 2′-H, 3′-H, 4′-CH_3 (all <0.3)
2-H	5.63 d	3-H (4.3)
3-H	4.80 m	2-H (4.3), NH (8.2), 4-H_a (8.6), 4-H_b (10.6)
OH[c]	4.26 br s	
6-H	3.68 dd	7-H (3.5), 10-H (3.9)
10-H	3.57, 3.64 dd	6-H (3.9), 9-H (3.5)
9-H	3.46 dd	7-H (2.9), 10-H (3.5)
7-H	3.44, 3.39 dd	6-H (3.5), 9-H (2.9)
4-H_a	2.65 dd	3-H (8.6), 4-H_b (13.0)
6′-H	2.52 m	5′-H (9.4), 6′-CH_3 (6.7), 7′-H_a, 7′-H_b (9.4)
4-H_b	2.06 dd	3-H (10.6), 4-H_a (13.0)
4′-CH_3	1.78 d	5′-H (<0.3)
7′-H_a	1.36 m	6′-H, 7′-H_b, 8′-2H
7′-H_b	1.26 m	6′-H, 7′-H_a, 8′-2H
8′-2H···11′-2H	1.31~1.18 m	7′-H_a, 7′-H_b, 12′-3H
6′-CH_3	0.98 d	6′-H (6.7)
12′-3H	0.88 t	11′-2H (6.8)

[a] Sample concentration 5 mg/ml; at a concentration of 50 mg/ml slight chemical shift variations occurred (second ppm value) which led to a separation of the otherwise overlapping 7-H and 9-H signals.
[b] Only those values are given which could be clearly determined.
[c] Exchanges with D_2O.

measured. The crucial part of this spectrum is presented in Fig. 2. The course of arrows gives the correlations *via* one bond C-C couplings and thus the complete carbon atom sequence from C-2 to C-10 including the branching at C-5. In the same way the total carbon skeleton of aranorosin could be derived from these data (see last column of Table 2) thus proving its constitutional formula.

Stereochemistry

Since the evaluation of the ^1H-^1H couplings did not lead to unequivocal assignments of the relative configuration at the various stereochemical centers in aranorosin, a series of selective nuclear Overhauser effect (NOE) measurements was performed. The results are summarised in Table 3.

The 15.3 Hz coupling of the olefinic protons (Table 1) indicated the 2′,3′ double bond to have *E* configuration. This was further substantiated by a NOE between 2′-H and 4′-CH_3. Further NOE's between 4′-CH_3 and 6′-H as well as 3′-H and 5′-H established an *E* configuration for the 4′,5′ double bond too, and, in addition, the conformation of the diene system as depicted in Fig. 3. Despite the fact that the C-5′, C-6′ bond seems to have a preferred conformation (5′-H and 6′-H anti-periplanar as in Fig. 3), no differentiation of the methyl and the hexyl groups attached to C-6′ could be obtained. So the relative configuration of C-6′ could not be determined.

From angular constraints drawn from the ^1H-^1H couplings and from distance constraints obtained from the NOE experiments, the stereochemistry of the spiro ring system in aranorosin could be derived (Fig. 4). NOE's indicate that 2-H, 3-H and 4-H are *cis*-oriented. The ^1H-^1H couplings in the hemi-

Table 2. ^{13}C NMR spectroscopic data of aranorosin in $CDCl_3$.

Carbon atom	Chemical shift (ppm)	Multiplicity[a]	Coupled protons		Coupled carbons
			Direct C-H coupling[b]	Long range C-H coupling[c]	
C-8	198.4	s	—	7-H, 9-H	C-7, C-9
C-1′	167.0	s	—	NH, 2′-H, 3′-H	C-2′
C-5′	148.5	d	5′-H	4′-CH_3, 6′-CH_3	C-4′, C-6′
C-3′	147.4	d	3′-H	4′-CH_3, 5′-H	C-2′, C-4′
C-4′	130.8	s	—	2′-H, 4′-CH_3	C-3′, 4′-CH_3, C-5′
C-2′	117.0	d	2′-H	NH	C-1′, C-3′
C-2	96.6	d	2-H	—	C-3
C-5	78.8	s	—	2-H, 10-H	C-4, C-6, C-10
C-6	64.4	d	6-H	4-H_b	C-5, C-7
C-10	63.0	d	10-H	4-H_a, 4-H_b, 9-H	C-5, C-9
C-7	55.8	d	7-H	6-H, 9-H, 10-H	C-6, C-8
C-9	55.6	d	9-H	7-H, 10-H	C-8, C-10
C-3	52.0	d	3-H	—	C-2, C-4
C-7′	37.2	t	7′-H_a, 7′-H_b	6′-H, 6′-CH_3	C-6′, C-8′
C-4	35.9	t	4-H_a, 4-H_b	—	C-3, C-5
C-6′	33.2	d	6′-H	5′-H, 8′-2H	C-5′, 6′-CH_3, C-7′
C-10′	31.8	t	(10′-2H)[d]	(9′-2H, 11′-2H)[d], 12′-3H	C-9′, C-11′
C-9′	29.4	t	(9′-2H)[d]	—	C-8′, C-10′
C-8′	27.4	t	(8′-2H)[d]	—	C-7′, C-9′
C-11′	22.6	t	(11′-2H)[d]	12′-3H	C-10′, C-12′
6′-CH_3	20.5	q	6′-CH_3	—	C-6′
C-12′	14.1	q	12′-3H	—	C-11′
4′-CH_3	12.4	q	4′-CH_3	3′-H, 5′-H	C-4′

[a] Determined using DEPT-135 and fully coupled spectra.
[b] Optimized for $^1J_{CH} \simeq 135$ Hz.
[c] Optimized for $J_{CH} \simeq 20$, 12.5, 8 and 5 Hz; details see Experimental.
[d] Since 8′-2H···11′-2H appear as overlapping signals individual assignments could not be made directly from the spectra.

Fig. 2. Contour plot of the 2D INADEQUATE spectrum of aranorosin (I), establishing the carbon-carbon connectivities in the spiro system.

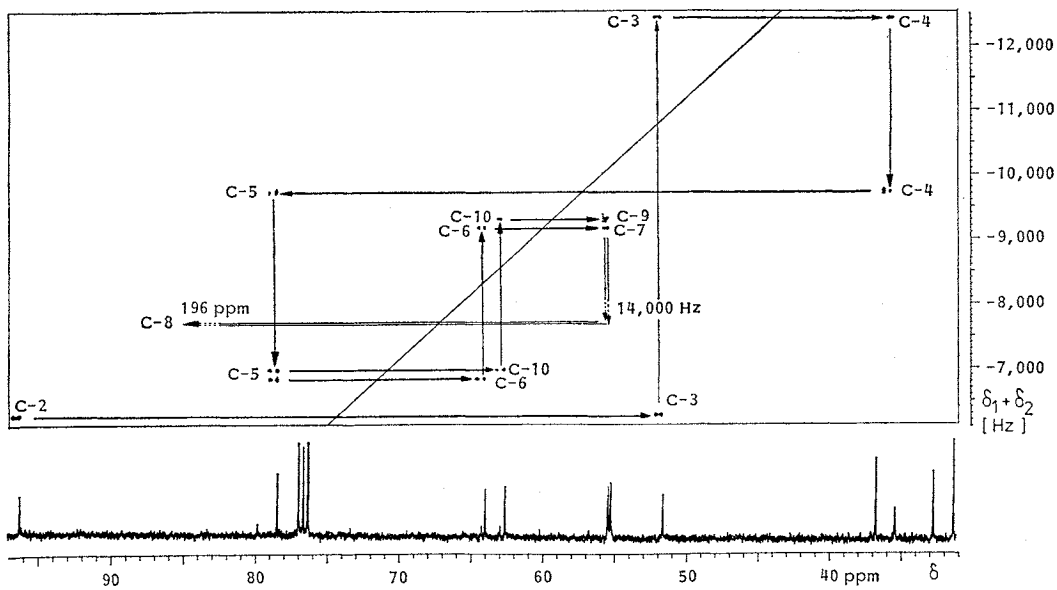


Table 3. NOE studies of aranorosin.

Irradiated proton	Response (% intensity enhancement)
2-H	3-H (2.1)
3-H	2-H (3.0), 4-H_a (2.3), 10-H (3.7)
4-H_a	3-H (2.1), 4-H_b (7.0), 10-H (2.8), 9-H (2.2)
4-H_b	4-H_a (7.7), 6-H (2.8)
6-H	4-H_b (1.6), 7-H (3.9)
7-H	6-H (1.6)
9-H	10-H (2.5)
10-H	3-H (2.4), 4-H_a (1.8), 9-H (2.5)
4'-CH_3	2'-H (4.8), 6'-H (5.4)
5'-H	3'-H (10.9)
6'-H	4'-CH_3 (5.2)

Fig. 3. Sterical arrangement of the double bond system showing spatial proximity of protons as established by NOE's (arrows).

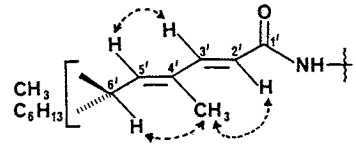
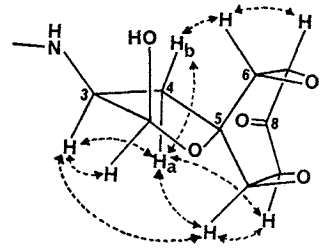


Fig. 4. Relative stereochemistry of the spiro ring system (NOE's indicated by arrows).



acetal ring, especially the two large couplings between 3-H and both 4-H_a and 4-H_b (see Table 1), suggest dihedral angles of $\leq 40°$ for 2-H/3-H, $\leq 20°$ for 3-H/4-H_a and $\geq 160°$ for 3-H/4-H_b which severely restrict the conformation of the five membered ring. Rather large four-bond couplings between the epoxide protons (6-H, 10-H, $J=3.9$ Hz; 7-H, 9-H, $J=2.9$ Hz) indicate coplanarity of the corresponding bond systems resulting in a boat conformation of the six membered ring with both oxygens on the same side. Additional NOE's showed spatial proximity between 4-H_a and 9-H and 10-H as well as between 4-H_b and 6-H. Hence these must be on the same side of the C-6/C-7/C-9/C-10 plane; in other words, the two epoxide groups and the hemiacetal oxygen are on the same side.

Experimental

All mp's are uncorrected. UV spectra were recorded using a Uvikon 810 double beam spectrophotometer. IR spectra were recorded on a Perkin-Elmer 157 sodium chloride spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Jeol FX 90Q, Bruker AM-270 and AM-400 spectrometers and mass spectra on an MS 902 S (Kratos). Optical rotation was measured on a Perkin-Elmer 141 polarimeter.

Aranorosin (I)

The isolation of aranorosin (I) has been reported earlier[1]. It was obtained as colorless crystalline solid from EtOAc - petroleum ether: MP 150°C (dec); $[\alpha]_D^{20}$ −2.42° (*c* 2.58, $CHCl_3$); HR-MS $C_{23}H_{33}NO_6$ found *m/z* 419.228, calcd 419.231.

Anal Calcd for $C_{23}H_{33}NO_6 \cdot \frac{1}{2}H_2O$: C 64.48, H 7.94, N 3.27.
Found: C 64.75, H 7.83, N 3.16.

UV λ_{max}^{MeOH} nm 264; no alkali or acid shift; IR ν_{max}^{KBr} cm^{-1} 3460, 1724, 1710, 1650, 1613, 1525, 1455, 1242, 1025, 980, 930, 880, 790; ^1H NMR spectrum see Table 1; ^{13}C NMR spectrum see Table 2; MS see Fig. 1.

Aranorosin Monoacetate (II)

Aranorosin (I, 100 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (2 ml) was added. The reaction mixture was kept at room temperature for 24 hours and then poured into ice water. The solid obtained was filtered, washed with 1 N $NaHCO_3$, water and dried. The product was purified by column chromatography over silica gel (200~300 mesh) using 1% MeOH in $CHCl_3$ for elution. Yield: 80 mg.

Table 4. ^{1}H NMR data of aranorosin derivatives in $CDCl_3$.

Proton	Chemical shift (ppm) (multiplicity, coupling constant in Hz)				
	II[a]	III[b]	V[a]	VI[b]	VII[b]
2-H	6.51 (d, 4)	—	6.38 (d, 4)	—	—
3-H	5.03 (m)	4.72 (dd, 10.5, 9)	5.08 (m)	—	—
4-H_a	2.63 (dd, 12.5, 8.5)	*	2.65 (dd, 12.5, 8.5)	—	—
4-H_b	2.04 (dd, 12.5, 10.5)	*	2.20 (dd, 12.5, 10.5)	—	—
6-H	3.49 (t, 3.5)[c]	3.74 (m)[c]	6.89 (d, 2.5)[d]	—	—
10-H	3.57 (t, 3.5)[c]	3.97 (m)[c]	7.06 (d, 2.5)[d]	—	—
7-H and 9-H	3.46 (m)	3.52 (m)	—	—	—
2′-H	5.73 (d, 15)	5.80 (d, 14.5)	5.74 (d, 15)	5.75 (d, 15)	5.77 (d, 15)
3′-H	7.30 (d, 15)	7.22 (d, 14.5)	7.31 (d, 15)	7.37 (d, 15)	7.32 (d, 15)
4′-CH_3	1.78 (br s)	1.76 (d, 1)	1.80 (br s)	1.78 (br s)	1.76 (br s)
5′-H	5.71 (d, 10)	5.64 (d, 10)	5.72 (d, 10)	5.70 (d, 10)	5.65 (d, 10)
6′-H	2.53 (m)	*	2.52 (m)	2.50 (m)	2.48 (m)
6′-CH_3	0.98 (d, 7)	0.97 (d, 7)	0.98 (d, 7)	0.98 (d, 8)	0.96 (d, 8)
7′-2H··· 11′-2H	1.23 (m)	1.24 (m)	1.26 (m)	1.25 (m)	1.24 (m)
12′-3H	0.86 (t, 6)	0.92 (t, 6)	0.87 (t, 6)	0.96 (t, 7)	0.92 (t, 7)
NH	5.64 (d, 8)	6.70 (d, 7)	5.61 (d, 8)	—	—
$OCOCH_3$	2.19 (s)	—	2.24 (s)	—	—
$COOCH_3$	—	—	—	—	3.72 (s)

[a] Recorded at 270 MHz.
[b] Recorded at 90 MHz.
[c] Assignments are interchangeable.
[d] This assignment is not unambiguous. It may be due to 7-H and 9-H. See text.
* 2.42~3.0 (m, 3H).

The monoacetate (II) is a colorless crystalline solid obtained from $CHCl_3$ - petroleum ether: MP 108~110°C; MS $C_{25}H_{35}NO_7$, M^+ *m/z* 461; UV λ_{max}^{MeOH} nm 265; IR ν_{max}^{KBr} cm^{-1} 3448, 3030, 1754, 1667, 1639, 1538, 1460, 1379, 1351, 1235, 1117, 1070, 1010, 962, 930, 877, 790; ^{1}H NMR see Table 4.

Oxidation of Aranorosin to the Lactone (III)

Aranorosin (I, 100 mg) was dissolved in CH_2Cl_2 (10 ml) and pyridinium dichromate (600 mg) was added. The reaction mixture was stirred at room temperature for 24 hours. It was then poured into water and extracted with CH_2Cl_2 (2×20 ml). The combined CH_2Cl_2 extracts were washed with water (2×20 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel (200~300 mesh) using 1% MeOH in $CHCl_3$ for elution. Yield: 10 mg.

Aranorosin (I) was also oxidized using JONES reagent[3]. The compound (I, 100 mg) was dissolved in acetone (5 ml) and JONES reagent was added dropwise until a reddish-brown color persisted. The reaction mixture was then poured into water and extracted with ether (3×20 ml). The combined ether extracts were washed with 0.1 N $NaHCO_3$ (1×20 ml) and water (2×20 ml), dried over anhydrous Na_2CO_3 and evaporated under reduced pressure. The crude product was purified by Medium Pressure Liquid Chromatography (using silica gel (200~300 mesh), solvent: 5% MeOH in $CHCl_3$, flow rate: 30 ml/minute). Yield: 25 mg.

The γ-lactone (III) was obtained as white solid from $CHCl_3$ - petroleum ether: MP 175~178°C; MS $C_{23}H_{31}NO_6$, M^+ *m/z* 417; UV λ_{max}^{MeOH} nm 265; IR ν_{max}^{KBr} cm^{-1} 3500, 2985, 1786, 1724, 1667, 1626, 1504, 1250, 1200, 1087, 980, 940, 900, 847, 747; ^{13}C NMR (22.5 MHz, $CDCl_3$+2 drops of DMSO-d_6) δ 12.46, 13.98, 20.37, 22.54, 27.42, 29.37, 31.75, 33.16, 33.59, 37.17, 48.98, 55.16, 61.44, 61.77, 78.24, 116.81, 131.01, 147.37, 148.24, 167.20, 173.81, 197.32; ^{1}H NMR see Table 4.

Preparation of IV and V

Aranorosin (I, 200 mg) was added to pyridinium chloride in pyridine (3 equiv) and stirred at room temperature for 16 hours. The reaction mixture was then poured into cold water and extracted with $CHCl_3$ (3×50 ml). The combined $CHCl_3$ extracts were washed with 1 N HCl (2×40 ml) and water (2×40 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude di(chlorohydrin) (IV) was purified by column chromatography over silica gel (200~300 mesh) using 2% MeOH in $CHCl_3$ for elution. Yield: 100 mg.

The compound (IV) was obtained as colorless crystalline solid from $CHCl_3$ - petroleum ether: MP 118~119°C; $C_{23}H_{35}NO_6Cl_2$;

Anal Calcd for $C_{23}H_{35}NO_6Cl_2\cdot H_2O$: C 54.22, H 7.26, N 2.75, Cl 13.75.
Found: C 54.42, H 8.02, N 2.33, Cl 14.16.

IR λ_{max}^{KBr} cm^{-1} 3450, 2985, 1653, 1613, 1527, 1450, 1342, 1290, 1220, 1130, 1031, 985, 845, 787; MS *m/z* 473 (M^+-H_2O).

Compound IV (90 mg) was acetylated using acetic anhydride (3 ml) and pyridine (0.5 ml) at room temperature for 24 hours. On usual work up, the acetate (V) was obtained as slightly impure solid, which was purified by column chromatography over silica gel (200~300 mesh) using 0.5% MeOH in $CHCl_3$ for elution. Yield: 60 mg.

The acetate (V) was obtained as colorless crystalline solid from $CHCl_3$ - petroleum ether: MP 174~177°C; MS $C_{25}H_{33}NO_5Cl_2$, M^+ *m/z* 497; UV λ_{max}^{MeOH} nm 260; IR ν_{max}^{KBr} cm^{-1} 3333, 2900, 1740, 1680, 1639, 1600, 1515, 1450, 1361, 1316, 1220, 970, 900, 833, 781, 772; ^1H NMR see Table 4.

Reaction of Aranorosin (I) with Zn - Ac_2O - CH_3COONa Followed by CH_2N_2

Aranorosin (I, 100 mg) was dissolved in acetic anhydride (10 ml) and freshly fused sodium acetate (100 mg) was added. The reaction mixture was refluxed for 30 minutes and zinc (500 mg) was added. The refluxing was continued for another 10 minutes and then the reaction mixture was poured into ice water. The aqueous solution was extracted with $CHCl_3$ (3×20 ml) and the combined $CHCl_3$ extract were washed with water (2×20 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The gummy residue was chromatographed over silica gel (200~300 mesh) using 0.1% MeOH in $CHCl_3$ for elution. Only one product could be isolated in minor amounts from the complex mixture. Yield: 6 mg. The product, identified as the side chain carboxylic acid (VI) of aranorosin, was obtained as gum.

The acid (VI, 5 mg), recovered after taking a ^1H NMR spectrum, was added to CH_2N_2 - ether (2 equiv) and kept at 0°C for 24 hours. The solvent was evaporated to give the methyl ester (VII) as a white solid from ether - petroleum ether. Yield: 5 mg.

^1H NMR data of VI and VII see Table 4. Further data on VI and VII could not be obtained owing to the lack of material.

NMR Experiments

One- and two-dimensional NMR measurements were performed with a Bruker AM-400 WB spectrometer, equipped with an Aspect 3000 computer. ^1H NMR spectra (400.1 MHz) were recorded from solutions containing either 5 mg/ml or 50 mg/ml sample in $CDCl_3$. The spectral width was 3,200 Hz, 32 k data points without zero-filling. ^{13}C NMR spectra (100.6 MHz) were recorded from a 50-mg/ml solution in $CDCl_3$. The spectral width was 20,000 Hz, 32 k data points and zero-filling, using quadrature-detection and broad-band proton decoupling.

For ^1H-^1H correlation (COSY), the sequence 90°-t_1-90°-acquisition was performed. Relaxation delay 0.5 second, a 90° pulse of 6.3 μseconds was used. The acquisition time was 853 mseconds, spectral width in F1 and F2 was 2,800 Hz, 1,024 experiments with either 64 (5 mg sample) or 16 (50 mg sample) scans of 4,096 points were performed. The data points in F1 were zero-filled to give a $2,048\times2,048$ data matrix. In both dimensions a shifted sine-bell apodization was used.

For ^{13}C-^1H correlation the sequence 90° (^1H)-$t_1/2$-180° (^{13}C)-$t_1/2$-Δ_1-90° (^1H, ^{13}C)-Δ_2-acquisition was performed with a 50-mg/ml solution and 5 mm sample diameter. The 90° pulse was 8.0 μseconds for ^1H and 5.9 μseconds for ^{13}C, relaxation time 1.6 seconds. To establish the correlation for selective polarization-transfer *via* the 135 Hz mean direct coupling, $\Delta_1=3.7$ and $\Delta_2=1.8$ mseconds were chosen.

The spectral width was 20,000 Hz in F2 and 2,800 Hz in F1. 512 experiments with 128 scans of 4,096 data points were performed. The acquisition time was 102.4 mseconds. After exponential multiplication (4 Hz line broadening) in F2 and sine-bell apodization in F1, zero-filling in both dimensions, the Fourier transformation gave a spectrum of 1,024×4,096 real data points.

For the correlations *via* small couplings several spectra were recorded, Δ_1 and Δ_2 were chosen appropriately for mean coupling constants of 20 Hz (Δ_1=25, Δ_2=12.5 mseconds), 12.5 Hz (Δ_1=40, Δ_2=20 mseconds), 8 Hz (Δ_1=62.5, Δ_2=31.3 mseconds) and 5 Hz (Δ_1=100, Δ_2=50 mseconds). 256 experiments with 256 scans of 4,096 data points were taken. All other parameters remained the same as in the experiment for the one-bond coupling.

For ^{13}C-^{13}C correlation (INADEQUATE), the sequence 90° (^{13}C)-Δ_1-180° (^{13}C)-Δ_1-90°(^{13}C)-t_1-90°(^{13}C)-acquisition was applied on a saturated solution (100 mg/ml, 10 mm sample tube) in $CDCl_3$. The 90° pulse length was 11.0 μseconds. To generate double quantum coherence, Δ_1 was chosen appropriate to a mean carbon-carbon coupling constant of 55 Hz (Δ_1=4.5 mseconds). The spectral width was 20,000 Hz in F2 and 18,000 Hz in F1. 1,024 experiments with 1,024 scans of 4,096 data points were performed. The acquisition time was 102.4 mseconds. After exponential multiplication (10 Hz line broadening) in both dimensions and zero-filling, the Fourier transformation gave a spectrum of 2,048×4,096 real data points.

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