Structures of the Actaplanins

Ann H. Hunt,* Thomas K. Elzey, Kurt E. Merkel, and Manuel Debono

Lilly Research Laboratories and Biochemical Development Division, Eli Lilly and Company, Indianapolis, Indiana 46285

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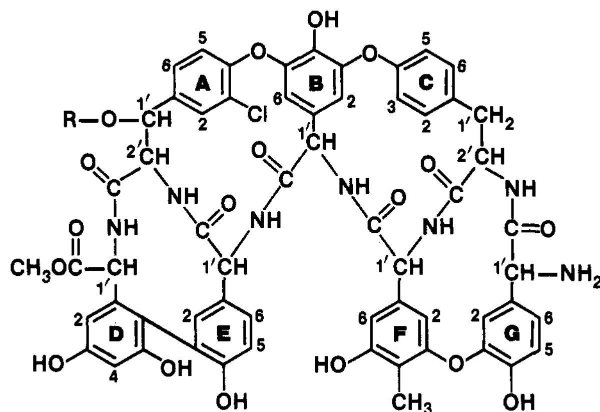
The actaplanins are a family of glycopeptide antibiotics having a common aglycon but differing in their content and/or distribution of attached sugar units. Sites of sugar attachment in the actaplanins have been determined by NMR methods, including the observations of negative nuclear Overhauser effects. These results, when combined with information from the analysis of sugar content and from the observation of common acidic degradation pathways, have led to an explanation of the structures of the various actaplanin glycopeptides. All of the actaplanins contain ristosamine attached to the aglycon via a benzylic hydroxyl group, and they all contain either one or two mannose moieties, attached as monosaccharides at phenolic sites. An additional phenolic site in each actaplanin is occupied by either glucose, mannosylglucose, or rhamnosylglucose.

Introduction

Actaplanin is a complex of glycopeptide antibiotics produced by *Actinoplanes missouriensis*; the actaplanins, like other members of this class, have an amino sugar and several neutral sugars attached to a peptide core of aromatic amino acids. Complete structures have been reported for three members of this class of antibiotics: vancomycin,[1] ristocetin A,[2-4] and avoparcin.[5]

Structures have been determined for the pseudoaglycons (peptide core plus amino sugar) of two additional glycopeptide antibiotics, A35512B[6] and actaplanin.[7] The ψ-aglycon 1 is common to all the actaplanin glycopeptides;[8]



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the actaplanins differ only in the nature and/or distribution of the neutral sugar moieties attached to the peptide core. The sugar content has been determined for actaplanins A, B_1, B_2, B_3, C_1, and G;[8] these antibiotics have up to four neutral sugars in combinations containing glucose, rhamnose, and/or mannose, as shown in Table I. Determining how the sugar units are attached to 1 in the various members of the actaplanin family is the subject of this paper. The structural relationships among the actaplanins have been deduced, based on observation of acidic degradation pathways, isolation and identification of hydrolysis intermediates, and determination of the sites of sugar attachment by NMR methods.

Table I. Neutral Sugar Content of Six Actaplanins[a]

actaplanin	glucose	mannose	rhamnose
A	1	3	
B_1	1	2	1
B_2	1	2	
B_3	1	2	
C_1	1	1	1
G	1	1	

[a] From Table II of ref 8.

Experimental Section

NMR Studies. Proton NMR spectra of the various actaplanins were recorded by using a Bruker WH360 spectrometer in the Fourier transform mode. Solutions were prepared in either Me_2SO-d_6 or Me_2SO-d_6 containing D_2O; the sample temperature during data acquisition was usually either ambient (~23 °C, used for all difference NOE experiments and for decoupling studies on some of the actaplanins) or 60 °C (used for most decoupling studies). Difference NOE (nuclear Overhauser effect) spectra were obtained by subtracting free induction decays accumulated with the decoupler off-resonance from similar accumulations with particular resonances irradiated, followed by Fourier transformation of the difference signals. The procedure was not optimized for maximum NOE measurement; the usual irradiation period was 2.0 s, followed by a preaccumulation delay of 0.03 s.

Isolation and Chromatographic Separation of the Actaplanins. The isolation of the actaplanin complex and the separation of the actaplanins by polyamide chromatography was reported previously.[8] Additional purification of the individual factors was achieved by rechromatography through C-18 reversed-phase silica. The actaplanins were eluted with aqueous acetonitrile gradients containing triethylamine phosphate. The triethylamine phosphate was removed from the concentrated chromatography fractions by readsorption on C-18 bonded phase and elution of the actaplanins with aqueous methanol. The methanol was removed by evaporation, and the resulting aqueous solutions were freeze-dried.

The actaplanins C_2 and D_1 were obtained from the partial hydrolysis mixtures of factors (A, B_1, and B_2) and (B_3, C_1, and G), respectively. The hydrolysis mixtures were separated by low-pressure chromatography using C-18 reversed-phase silica.

Acidic Hydrolysis of the Actaplanins. Aqueous solutions containing (0.5 mg actaplanin)/mL were acidified with aqueous

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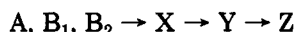
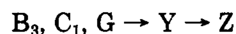
HCl to pH 1.85. The acidic actaplanin solutions were heated on a steam bath for a fixed length of time and then were allowed to cool to room temperature. The cooled solutions were analyzed directly by high-performance liquid chromatography, using absorbance detection at 254 nm.

Results

The six actaplanins listed in Table I can be divided into two groups on the basis of their behavior when subjected to mild acid hydrolysis.[8] Actaplanins A, B_1, and B_2 give rise to three major degradation products in sequence (X, Y, and Z); actaplanins B_3, C_1, and G, however, are hydrolyzed to Y and then Z but never produce X:



$$A, B_1, B_2 \rightarrow X \rightarrow Y \rightarrow Z$$



$$B_3, C_1, G \rightarrow Y \rightarrow Z$$

The final hydrolysis product, Z, is the actaplanin ψ-aglycon 1.[7,8] The existence of two major degradation pathways for the six actaplanin glycopeptides suggests that distinct structural relationships exist among the members of the group. The first step in learning what these relationships are was to determine the sites of sugar attachment to the aglycon by NMR methods, using NOE observations and also developing chemical shift correlations. The second step in the structure elucidation sequence was to isolate and characterize the hydrolysis intermediates X and Y. The third step in solving the actaplanin structures was to devise a hydrolysis scheme consistent with all the experimental evidence, including the existence of minor hydrolysis pathways leading to intermediates other than X and Y. The results of these steps will be discussed in the order listed above.

Sites of Sugar Attachment

The approach used in attacking the question of the sites of sugar linkage to the ψ-aglycon was to look for nuclear Overhauser effects—either NOEs involving free phenols and aromatic protons adjacent to them or NOEs involving the anomeric protons of sugars directly attached to 1 and aromatic protons adjacent to the attachment site. The actaplanins were studied approximately in order of their increasing complexity; the first ones to be examined were actaplanins C_3, G, and B_2.

Actaplanin C_3. The ^{1}H NMR spectrum of this glycopeptide contains four phenolic OH resonances (cf. six phenols in 1) and only two sugar anomeric resonances; it was chosen for NMR study on the basis of this simplicity. Although neither the sugar content nor the hydrolysis pathway of actaplanin C_3 has been examined, it will be shown in subsequent sections of this paper that C_3 must contain glucose and mannose and that it will produce neither X nor Y upon hydrolysis.

The ^{1}H NMR assignments for the aglycon and sugar anomeric protons for actaplanin C_3 are given in Table II; the assignments are based on decoupling studies and NOE observations similar to those described for the actaplanin ψ-aglycon.[7] The four phenolic peaks were assigned by irradiating them in difference NOE experiments, with the following NOEs observed: irradiation of a phenol at 8.88 ppm produced a negative NOE at a 6.42 ppm resonance (D-4), while irradiation of the phenol at 9.52 ppm produces NOEs at both D-4 and D-2 (6.09 ppm); these two phenols must be at positions D-5 and D-3, respectively. Irradiation of the phenolic resonance at 9.33 ppm causes negative NOE to a doublet at 6.72 ppm (E-5), identifying this as the phenol at position E-4. The last phenolic resonance (at 9.98 ppm) is broad and difficult to identify, but a weak NOE to a doublet at 7.07 ppm (G-5) was observed after

Table II. Chemical Shifts (δ) for Aglycon and Anomeric Protons for Several Actaplanins in Me_2SO[a]

proton	ψ[b]	C_3[c]	G[d]	B_2[e]
G-4	9.98	9.98		
F-5	9.57		9.56	
D-3	9.51	9.52	9.78	9.75
B-4	9.47			
E-4	9.31	9.33	9.49	9.50
D-NH	9.05	8.98	9.35	9.31
D-5	8.97	8.88		
E-NH	8.57	8.53	8.70	8.75
C-NH	7.98	7.89	7.92	7.94
C-2	7.85	7.82	7.85	7.80
A-2	7.70	7.72	7.71	7.71
F-NH	7.62	7.64	7.55	7.58
B-NH	7.60	7.66	7.84	7.74
A-NH	7.49	7.40	7.51	7.37
A-6	7.33	7.32	7.34	7.33
A-5	7.31	7.32	7.34	7.33
C-5	7.19	7.22	7.22	7.19
E-2	7.19	7.22	7.13	7.15
G-6	7.19	7.22	7.13	7.19
G-5	7.09	7.07	7.00	7.05
C-6	7.05	7.10	7.09	7.09
C-3	6.90	7.01	6.90	7.00
E-6	6.74	6.74	6.79	6.78
G-2	6.72	6.73	6.56	6.62
E-5	6.69	6.72	6.77	6.76
D-4	6.42	6.42	6.79	6.81
F-2	6.40	6.66	6.41	6.64
F-6	6.38	6.68	6.39	6.72
D-2	6.06	6.09	6.26	6.29
B-2	5.63	5.73	5.73	5.77
B-1′	5.61	5.64	5.65	5.63
G-1′	5.51	5.29	5.24	5.29
Glu[f]		5.40	5.42	5.41
F-1′	5.27	5.38	5.20	5.35
Man[f]		5.26	5.30	5.31
Man[f]				5.31
A-1′	5.07	5.08	5.09	5.10
B-6	5.03	5.08	5.06	5.10
C-2′	4.92	4.94	4.79	4.88
Ris[f]	4.79	4.78	4.78	4.80
E-1′	4.49	4.53	4.60	4.61
D-1′	4.41	4.44	4.48	4.51
A-2′	4.26	4.27	4.23	4.29
OCH_3	3.73	3.69	3.70	3.71
C-1′	3.33	3.33	3.28	3.32
C-1′	2.85	2.88	2.87	2.88
$F\text{-}CH_3$	1.96	2.02	2.00	2.06

[a] Actaplanins C_3, G, and B_2 prepared by polyamide chromatography. [b] Actaplanin ψ-aglycon dihydrochloride, ~23 °C; data from ref 7. [c] 50 °C, except phenols ~23 °C. [d] ~23 °C. [e] ~60 °C, except phenols ~23 °C. [f] Anomeric protons of glucose (Glu), mannose (Man), or ristosamine (Ris).

taking a large number of accumulations in the difference NOE experiment. The 9.98-ppm resonance is therefore assigned to position G-4; the corresponding phenolic resonance was also difficult to assign in ristocetin A,[3] and it was missing from the spectrum in A35512B.[6]

The two anomeric resonances in the spectrum of actaplanin C_3 are a slightly broadened singlet at 5.26 ppm (sharpened by irradiation at 3.79 ppm) and a doublet at 5.40 ppm (collapsed by irradiation at 3.33 ppm). Mutual NOEs are observed between the 5.26-ppm resonance and the F-6 peak at 6.68 ppm, indicating that the F-5 phenol is a sugar attachment site in actaplanin C_3; the sugar attached to ring F will be shown in a subsequent portion of this report to be mannose. Linkage of a sugar to the aglycon through the F-5 phenol as also been demonstrated for A35512B.[6]

The remaining phenolic site in actaplanin C_3 is the B-4 phenol, which has no adjacent protons and thus no po-

Table III. Assignments for Actaplanin Aglycon Resonances between 6 and 7 ppm and for Neutral Sugar Anomeric Proton Resonances[a]

actaplanin	G-5 (d)	D-4	E-6 (d)	E-5 (d)	F-6	F-2	G-2	D-2	Glu (d)	Man	Rham
A	6.95	6.82*	6.80	6.74	6.76^{+}	6.61	6.52	6.28	5.70	5.31^{+}, 5.28*, 5.28	
B_1	6.93	6.81*	6.76	6.76	6.73^{+}	6.60	6.53	6.28	5.70	5.29^{+}, 5.29*	5.17
B_2	6.96	6.82*	6.78	6.76	6.74^{+}	6.62	6.55	6.27	5.43	5.29^{+}, 5.28*	
C_2	6.96	6.82*	6.80	6.74	6.75^{+}	6.63	6.54	6.28		5.31^{+}, 5.28*	
B_3	6.92	6.81*	6.81	6.75	6.42	6.36	6.51	6.29	5.70	5.27*, 5.27	
C_1	6.91	6.81*	6.77	6.75	6.41	6.36	6.50	6.28	5.70	5.27*	5.17
G	6.91	6.81*	6.76	6.76	6.41	6.37	6.50	6.28	5.42	5.27*	
D_1	6.92	6.81*	6.77	6.75	6.42	6.40	6.52	6.28		5.27*	

[a] Glycopeptides prepared by chromatography on C-18 reversed-phase silica. All resonances are singlets except where indicated; pairs of resonances marked by * or + exhibit mutual NOEs.

tential NOEs to be observed. It will be shown later, however, that the 5.40-ppm resonance must be the anomeric proton of a glucose moiety attached to the aglycon at B-4.

Actaplanin G. Assignments for the aglycon and sugar anomeric protons of actaplanin G are also give in Table II; this glycopeptide also contains only two sugars, glucose and mannose (see Table I).[8] There are only three distinct phenolic resonances in the spectrum of actaplanin G; these resonances were assigned to phenols at positions F-5, D-3, and E-4 by NOE observations of the same type as described for the phenols of actaplanin C_3. The mannose anomeric resonance in actaplanin G is a singlet at 5.30 ppm; it experiences mutual NOEs with the D-4 resonance at 6.79 ppm (one of the two which are reduced in intensity when the D-3 phenol is irradiated). For actaplanin G, therefore, the site of mannose linkage is the phenol at D-5 rather than on ring F; the D-5 phenol has a sugar attached in both ristocetin A (mannose)[2] and A35512B.[6]

Two phenolic sites (B-4 and G-4) and one anomeric resonance (glucose doublet at 5.42 ppm, coupled to a resonance at 3.34 ppm) remain to be considered for actaplanin G. There is no NOE evidence to show where the glucose is or is not attached, but analogy with actaplanin C_3 and other glycopeptides[1,2,6] suggests that the glucose is linked to the aglycon at B-4 (and thus there is no B-4 phenolic peak) and that the G-4 phenolic peak is missing from the spectrum due to exchange processes.

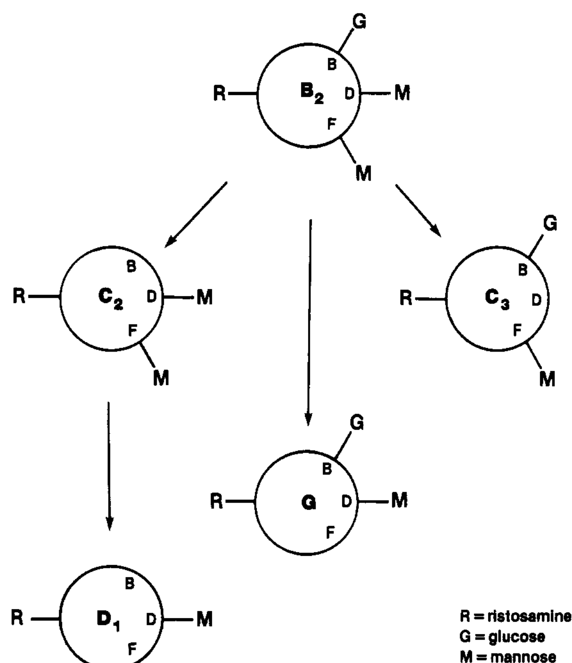
Actaplanin B_2. Table I lists three actaplanins containing only three neutral sugar moieties (B_2, B_3, C_1); the actaplanins B_2 and B_3 are identical in sugar content (one glucose and two mannoses)[8] but differ in sugar distribution. Actaplanin B_2 was the next glycopeptide of this family to be studied by NMR; the assignments are given in Table II. The spectrum of actaplanin B_2 contains only two distinct phenolic resonances (9.75 and 9.50 ppm); these were assigned by difference NOE observations to positions D-3 and E-4. The two mannose anomeric singlets are coincident at 5.31 ppm; NOEs are observed at 6.81 (D-4) and 6.72 ppm (F-6) when they are irradiated. Thus, mannoses are linked to both D-5 and F-5 sites in actaplanin B_2. The glucose anomeric resonance (doublet at 5.41 ppm, coupled to 3.36 ppm) again shows no participaton in NOEs; it is assumed for actaplanin B_2 that glucose is linked to the aglycon at B-4 and that the G-4 phenolic peak is missing. That this assumption is correct will eventually be demonstrated in the consideration of minor hydrolysis routes.

Chemical Shift Correlations. The NOE observations discussed above show that actaplanin B_2 has mannose units attached to 1 through the phenolic sites at both D-5 and F-5, while actaplanin G has mannose at D-5 (but not F-5) and actaplanin C_3 has a sugar (mannose) at F-5 but no sugar on ring D. When the chemical shifts of protons on the D and F rings are compared for the three actaplanins and for the ψ-aglycon (no neutral sugars), helpful correlatons emerge (see Table II). When the D-5 phenol is free (ψ and C_3), the D-2 and D-4 peaks occur near ~6.08 and 6.42 ppm; when a sugar is attached at D-5 (G and B_2), the peaks for D-2 and D-4 shift to ~6.28 and 6.80 ppm. Similarly, when the F-5 phenol is free (ψ and G), the F-2 and F-6 resonances are both near 6.40 ppm; when there is a sugar linkage at F-5 (C_3 and B_2), the F-2 resonance occurs near ~6.65 ppm and the F-6 peak near ~6.70 ppm. (The largest shift in each case is at the position adjacent to the sugar site; the lack of appreciable shifts for the G-5 protons of actaplanins G and B_2 supports the assumption that the G-4 phenol does not form a sugar linkage in either of these glycopeptides.)

These correlations are analogous to similar shifts observed between A35512B and the A35512B ψ-aglycon,[6] and they make the portion of an actaplanin spectrum between 6 and 7 ppm into a "fingerprint" for the presence of sugar linkages to rings D or F. Chemical shifts in this region of the spectrum are listed in Table III for a variety of these glycopeptides. The chemical shift correlations (supported in every case by NOE observations) indicate that actaplanins A, B_1, and B_2 all have sugars linked to the aglycon at both D-5 and F-5, while actaplanins B_3, C_1, and G each have a sugar at D-5 but a free phenol on ring F.

Structures of Hydrolysis Intermediates. The two major degradation intermediates observed when the actaplanins listed in Table I are subjected to mild acid hydrolysis have been isolated by using reversed-phase chromatography; they are actaplanin C_2 (≡ hydrolysis product X) and actaplanin D_1 (≡ hydrolysis product Y). These two materials have been subjected to the same types of NMR examination that have been described for their predecessors, and relevant data concerning them have been included in Table III. Both C_2 and D_1 have only singlet sugar anomeric resonances; the first hydrolysis step in both of the major degradation pathways must involve the loss of glucose. Intermediate C_2 has two mannoses attached to the aglycon as manosaccharides at positions D-5 and F-5, while D_1 has a single mannose at D-5; the conversion of X into Y, therefore, occurs by hydrolysis of the bond linking mannose to ring F, while repetition of this step at ring D converts Y into Z (ψ-aglycon). The major degradation route involving actaplanins B_3, C_1, and G does not have product X as an intermediate because those three glycopeptides already lack a sugar on ring F; they are converted directly to Y by the same process that produces X from actaplanins A, B_1, and B_2. Actaplanin C_3, however, lacks the mannose on ring D and therefore cannot be hydrolyzed to either of the observed intermediates.

Scheme I.[a] Hydrolysis of Actaplanin B_2



[a] Boldfaced lettering inside each circle identifies the particular actaplanin; the smaller letters B, D, and F indicate sugar attachment sites at the phenolic positions B-4, D-5, and F-5, respectively.

Degradation Pathways

Actaplanins B_2 and G lose only glucose in the first major hydrolysis step, but A and B_3 must lose a disaccharide of glucose and mannose while actaplanins B_1 and C_1 must lose both glucose and rhamnose. It is reasonable to assume that the sugar units that are lost from the various actaplanins in the initial hydrolysis event were attached to the aglycon at a common site before hydrolysis, and suggestions have been presented earlier in this report that the remaining attachment site is the B-4 phenol of 1. A consideration of some minor hydrolysis routes will demonstrate that these assumptions are correct.

Actaplanin B_2 was shown in an earlier section of this paper to have two mannose moieties attached to 1 at D-5 and F-5 plus a glucose. The glucose could, in principle, either be attached directly to the aglycon at B-4 or be attached indirectly through one of the two mannoses. If the three sugars are all attached to 1 as monosaccharides, it should be possible to remove each one separately, producing three different two-sugar hydrolysis products. If the glucose is attached to a mannose, only two such products are possible; the linking mannose cannot be removed independently. When actaplanin B_2 was subjected to mild hydrolysis for 1 h and the resulting mixture was examined chromatographically, the main components present were B_2, C_2 (i.e., X, formed from B_2 by loss of glucose), and D_1 (i.e., Y, formed from C_2 by the loss of mannose). Two minor components were present, however. A peak corresponding to 3.4% of the total amount of glycopeptide present was identified as actaplanin G (formed by loss of mannose from position F-5), while approximately 1% of the total glycopeptide material had been converted to actaplanin C_3 (formed by loss of mannose from position D-5). These observations require that the structures of actaplanins B_2, C_2, C_3, D_1, and G be related as indicated in Scheme I. When actaplanin B_2 was hydrolyzed for 2.5 h, another trace component was observed (~0.5%); it does not correspond to any of the known actaplanins and must therefore have been formed by the loss of mannose from either of the intermediate hydrolysis products G or C_3. This compound (product O, Table V) was assigned a structure in which the only neutral sugar is glucose, attached to position B-4 of 1. The formation of this component is more pronounced in the direct hydrolysis of actaplanin G, where it is 2% of the total glycopeptide content present after 2.5 h of hydrolysis (see Table IV).

Table IV. Components Present After 2.5 h of Hydrolysis of Six Actaplanins[a]

components present	starting actaplanins, %					
	A	B_1	B_2	B_3	C_1	G
A	56	-	-	-	-	-
B_1	-	63	-	-	-	-
B_2	trace	7	19	-	-	-
B_3	6	-	-	55	-	-
C_1	-	3	-	-	32	-
G			3	2	9	21
K[b]	~4	-	-	-	-	-
L[b]	-	~2	-	-	-	-
C_3			~1	-	-	-
M[b]		-	-	4	-	-
N[b]	-		-	-	3	-
O[b]			~0.5		1	2
C_2	22	16	52	-	-	-
D_1	} 4	} 2	} 17	31	46	62
D_2[c]				-	-	-
ψ			2	5	6	9

[a] Samples were analyzed by HPLC after hydrolysis for 2.5 h in aqueous solution at pH 1.85 and 90 °C. No entry in a space indicates that the component was not reported; a dash (-) indicates that formation of that particular component by hydrolysis of the starting actaplanin is not possible. [b] These compounds have not been isolated separately, but their structures are assigned on the basis of hydrolysis origin and retention time (Table V). [c] Actaplanins D_1 and D_2 are isomers each having a single mannose attached at D-5 or F-5, respectively.

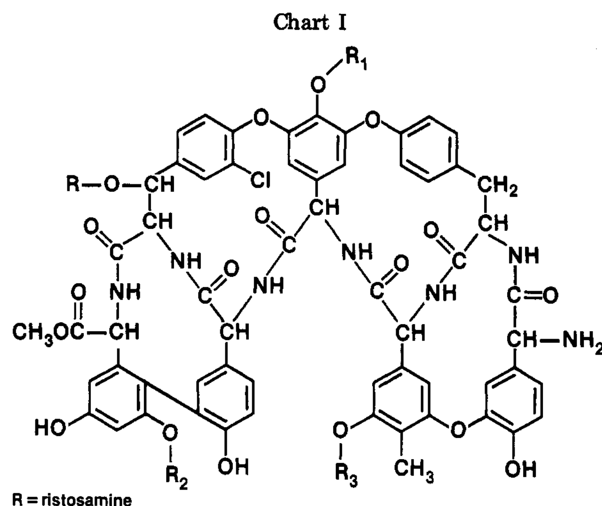
Table V. Structures of New Compounds Assigned on the Basis of Their Hydrolysis Origin and Their Retention by HPLC

compd	substituents on structure 2		
	R_1	R_2	R_3
D_2	H	H	mannose
K	mannosylglucose	H	mannose
L	rhamnosylglucose	H	mannose
M	mannosylglucose	H	H
N	rhamnosylglucose	H	H
O	glucose	H	H

Examination of the minor components produced by hydrolysis of actaplanins A and B_1 provides evidence that the glucosylmannose and glucosylrhamnose disaccharides are linked to the aglycon through glucose; both A and B_1 are converted to small extents to actaplanin B_2 by loss of either mannose or rhamnose. Actaplanins B_3 and C_1 are both converted to actaplanin G in minor amounts by analogous cleavages of their disaccharides.

The products observed after 2.5-h hydrolysis periods for actaplanins A, B_1, B_2, B_3, C_1, and G are summarized in Table IV. Loss of mannose from actaplanins A and B_1 can occur from either of the positions F-5 or D-5; two of the resulting compounds were identified as actaplanins B_3 and C_1 (loss of mannose from position F-5). The structures K and L (see Table V) were assigned to the two remaining compounds, formed by the loss of mannose from position D-5. The hydrolysis mixtures from actaplanins B_3 and C_1 also contained additional unknown components, and loss

Chart I



2

actaplanins	R_1	R_2	R_3
A	mannosylglucose	mannose	mannose
B_1	rhamnosylglucose	mannose	mannose
B_2	glucose	mannose	mannose
B_3	mannosylglucose	mannose	H
C_1	rhamnosylglucose	mannose	H
G	glucose	mannose	H
C_2 (≡ X)	H	mannose	mannose
D_1 (≡ Y)	H	mannose	H
ψ (≡ Z)	H	H	H
C_3	glucose	H	mannose

of mannose from D-5 is the only possibility for the formation of new hydrolysis products. Structures M and N (see Table V) were assigned to these components. The HPLC retention characteristics of compounds K, L, M, N, and O are consistent with their assigned structures and in agreement with the HPLC retention of other actaplanins.

The structural relationships among the various actaplanins are indicated by structure **2**; **2** is consistent with all the lines of evidence discussed in this paper (Chart I).

In the major hydrolysis pathways the actaplanins all lose the sugar R_1 as the first degradation step, converting A, B_1, and B_2 into C_2 and converting B_3, C_1, and G into D_1. Actaplanin C_2 is converted to D_1 by loss of R_3, and removal of R_2 from D_1 produces the ψ-aglycon **1**. Actaplanins G and C_3 are isomeric; it seems reasonable that isomers of B_3 and C_1 also exist and will be degraded (along with C_3) by a third hydrolysis pathway. Compounds K and L are the proposed isomers, but their hydrolysis characteristics have not been studied.

Conclusions

The complexity of the actaplanin family of glycopeptides arises from the large number of family members. The structural relationships are not complex, however, but are readily described by structure **2**. All actaplanins contain ristosamine in the ψ-aglycon **1**, and they all contain either one or two mannose moieties, attached as monosaccharides at phenolic sites. An additional phenolic site in each actaplanin is occupied by either glucose, mannosylglucose, or rhamnosylglucose.

Registry No. Actaplanin A, 88357-81-7; actaplanin B_1, 88357-82-8; actaplanin B_2, 88357-83-9; actaplanin B_3, 88357-84-0; actaplanin C_1, 88357-85-1; actaplanin G, 83381-73-1; actaplanin C_2, 88357-86-2; actaplanin D_1, 88357-87-3; actaplanin ψ, 88288-92-0; actaplanin C_3, 88357-88-4; actaplanin D_2, 88357-89-5; actaplanin K, 88357-90-8; actaplanin L, 88357-91-9; actaplanin M, 88357-92-0; actaplanin N, 88357-93-1; actaplanin O, 88357-94-2.

5,8-Quinoflavone. Synthesis and Addition Reactions

James H. Looker,* James R. Edman, and Charles A. Kingsbury

Department of Chemistry, The University of Nebraska, Lincoln, Nebraska 68588

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A synthesis of 5,8-quinoflavone (**3**) from primetin (**2**) is described. An improved Elbs persulfate oxidation procedure for **2** is presented. Addition of halogens and of hydrogen chloride to **3** is described, together with chemical and spectral data establishing the structure of the hydrogen chloride adduct (**4**) as 6-chloro-5,8-dihydroxyflavone. Chemical data include an independent synthesis of **4** via 6-chloro-5-hydroxyflavone (**9**). Methylation studies support the structural assignment given 2-methoxy-3-chloro-6-hydroxyacetophenone (**5**), from which **9** was synthesized in a four-step synthesis. Carbon-13 NMR spectral data for **4** are presented.

Quinones from flavonoid precursors have been reported by several workers.[1,2] In the present paper, we describe synthetic studies leading to primetin and 5,8-quinoflavone. Addition of halogens and hydrogen chloride to the quinone is described, together with data establishing the structure of the hydrogen chloride adduct.

Primetin (**2**) was obtained in 50% yield by a modification of the Elbs persulfate oxidation procedure,[3] in which tetraethylammonium hydroxide was the base. Removing oxygen from the reaction medium by nitrogen purging increased yields as much as 15%. Use of tetramethylammonium hydroxide in the oxidation gave yields of **2** under 10%, and tetra-n-propylammonium hydroxide and benzyltrimethylammonium hydroxide gave traces of **2**.

Primetin was oxidized to 5,8-quinoflavone (**3**) with lead tetraacetate in either benzene or acetic acid. The yield of **3** in the latter solvent was 68%, and in benzene 37%. For

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