URDAMYCINS, NEW ANGUCYCLINE ANTIBIOTICS FROM *STREPTOMYCES FRADIAE*

VI. STRUCTURE ELUCIDATION AND BIOSYNTHETIC INVESTIGATIONS ON URDAMYCIN H

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A new angucycline antibiotic has been discovered as a small side product of *Streptomyces fradiae* (strain Tü 2717), the producer of the urdamycin complex, during screening for biosynthetic relatives of urdamycins C and D.

The structure was elucidated after isolation, *via* strain selection, of a mutant of *S. fradiae* that produces this new congener in larger amounts.

The structure includes a new chromophore containing aglycone that has not been found before among the angucyclines nor as a natural product generally. In urdamycin H (**1**) the angucycline four-ring system is enlarged by a (*p*-OH-phenyl)furan moiety and is closely related to urdamycin C (**2**). The structure was elucidated by comparison of the physico-chemical data with those of known urdamycins, especially with those of urdamycin C (**2**), and was confirmed by intensive 2D NMR analysis.

Biosynthetic studies showed that tyrosine and not the smaller *p*-OH-phenylglycine is the precursor of the (*p*-OH-phenyl)furan moiety.

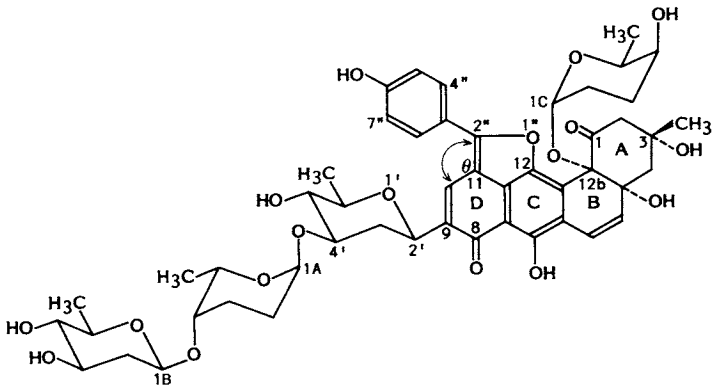
Angucycline antibiotics[1] were first discovered as anticancer as well as enzyme-inhibiting drugs.[2~5] Recently, new angucyclines were discovered during screening for platelet aggregation inhibitors.[6,7]

The urdamycins[8,9] are a complex of anticancer antibotics whose members differ mainly in the structure of their aglycones. Within the angucyclines they are the first members of this group found to contain enlarged aglycones, which also causes a dramatic change of the chromophore. Exemplifying this by having the most unusual chromophores are the urdamycins C (**2**) and D (**3**). While urdamycin A (**4**) has a hydroxynaphtoquinone chromophore that is often found in angucyclinones and thus shows an orange color, urdamycins C (**2**) and D (**3**) are characterized by their discoloration to dark red and blue, respectively.

Biosynthetic studies showed that the aglycone of urdamycin A (**4**) is formed by a single decaketide chain that forms the angucycline four-ring system, and the amino acids tyrosine and tryptophan enlarge the urdamycin A aglycone[10] thus leading to urdamycins C (**2**) and D (**3**), respectively. Especially the biomechanistic formation of these enlarged aglycones of urdamycins C (**2**) and D (**3**) continues to be the subject of our investigations.

One way to establish a mechanism of the aglycone enlargement is to find a relative of one of these antibiotics that is an intermediate between urdamycins A (**4**) and C (**2**). Thus we screened for further compounds with an enlarged angucycline aglycone (by looking for new red pigments signalizing a new chromophore) hoping to find such a key intermediate.

Upon examining the crude product of a 100-liter fermentation of *Streptomyces fradiae* (Tü 2717) we found a minor product (production *ca.* 0.05 mg/liter) with a lighter red color and a similar Rf value in comparison with urdamycin C. By strain selection we isolated a mutant capable of producing this



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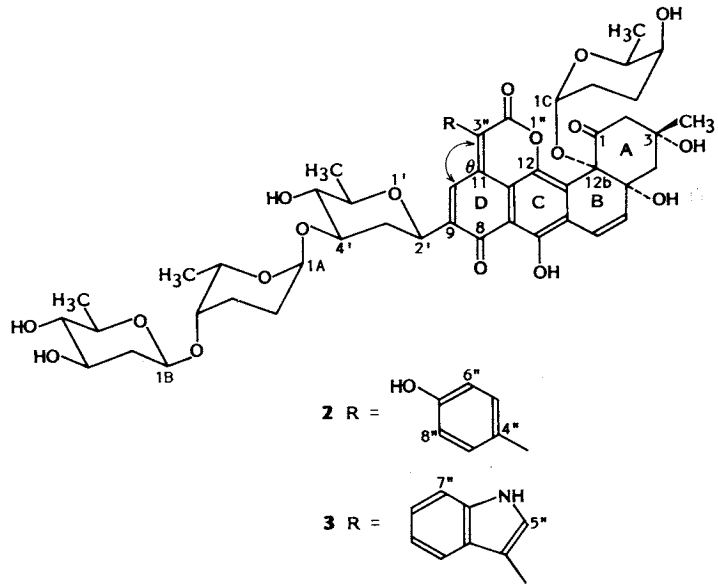

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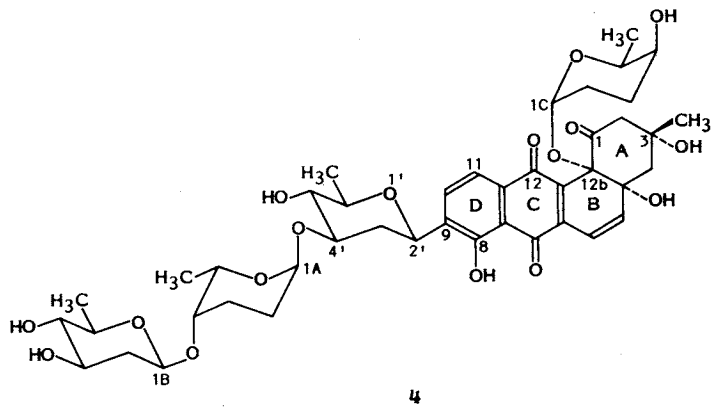

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metabolite in large amounts, *i.e.* 7 mg/liter. Comparison of the physico-chemical data for this new product with those of the other urdamycins (see below) showed that this compound is a new urdamycin, subsequently called urdamycin H. A relation with urdamycin C (**2**) was apparent from the NMR data. This hypothesis

was also confirmed by feeding [3,5-$^2H_2$]tyrosine which resulted in a 30%-incorporation into urdamycin H (see also below).

Physico-chemical Properties and Structure Elucidation

Urdamycin H (**1**) is an orange-red solid that is very soluble in acetone, lower alcohols or DMSO, sparingly soluble in chloroform, and insoluble in water or alkanes. In alkaline solutions the color changes to blue (indicator). The UV/visible and the CD spectra of urdamycin H are different from the spectra of all the other urdamycins and thus represent a new chromophore.

The fast atom bombardment (FAB)-MS show molecular ions at *m/z* 947 (negative FAB) and *m/z* 949 (positive ions) which is in accordance with a molecular formula of $C_{50}H_{60}O_{18}$ (948). This indicates a decrement of one C and one O in comparison with its closest relative, urdamycin C (**2**).

The IR spectrum of urdamycin H (Fig. 1) differs from that of urdamycin C (**2**) primarily in the much less intense absorption of the highest carbonyl band (1730 cm^{-1}) which indicates the absence of an ester (or lactone) C=O group.

The broad-band decoupled ^{13}C NMR spectrum of urdamycin H (**1**) in acetone shows 46 carbon signals, of which the signals at δ 67.4, 117.6, and 130.5 represent two carbons each (visible by heteronuclear correlation spectroscopy (HETCOR) analysis and deductible from the integration). An additional signal is obscured by the solvent, but can be detected *via* attached proton test (APT) or distortionless enhancement by polarization transfer (DEPT) analysis (δ 30.3). Comparison of the ^{13}C NMR data with those of urdamycin C (**2**) shows a wide agreement, *e.g.*, the *C*-glycosidic moiety, the sugars and the ring A carbons (see Table 1). Differences are evident in the rings C, D region, especially carbons 10, 11 and 11a. The isochronic carbon signals at δ 117.6 (two carbons) and δ 130.5 (two carbons) suggest a *p*-substituted phenyl residue, as in urdamycin C (see also Table 1). This hypothesis is confirmed by the ^1H NMR spectrum of **1** showing an aromatic AB system of two pairs of isochronic protons (δ 7.15 (2H) and 8.00 (2H, J=8 Hz each)). Furthermore, the ^1H NMR spectrum shows the same ring A and sugar proton signals as the other urdamycins. Differences are observable only in the downfield region, *i.e.* slight chemical shift differences of the olefinic AB system (δ 5.98 (5-H), 6.98 (J=10 Hz each, 6-H)); urdamycin C: δ 6.14 (5-H), 6.99 (J=10 Hz each, 6-H) and the marked downfield shift of 10-H (δ 8.38, d, J=2 Hz) in comparison with 10-H signal of urdamycin C (**2**, $\delta_{10\text{-H}}$ 7.89, d, J=2 Hz).

Thus a structural difference between urdamycins C and H due to the enlarged aglycone in the C-11/C-12 region could be assumed. The conclusion from the physico-chemical properties is a structure that is

Fig. 1. IR spectrum of urdamycin H (**1**) in KBr.

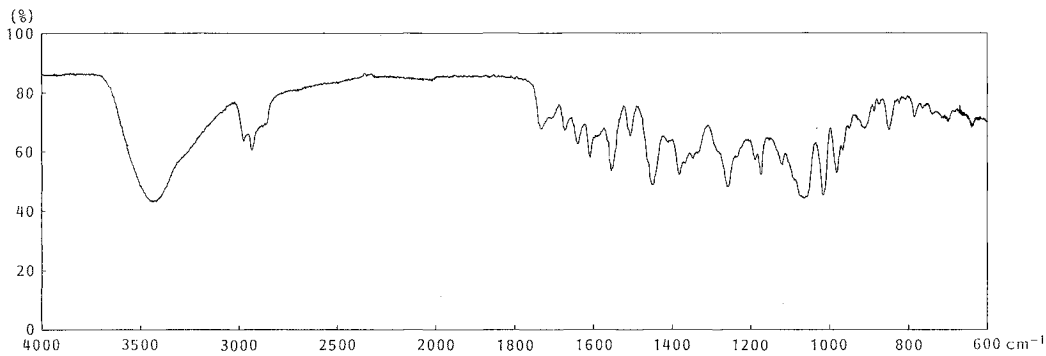


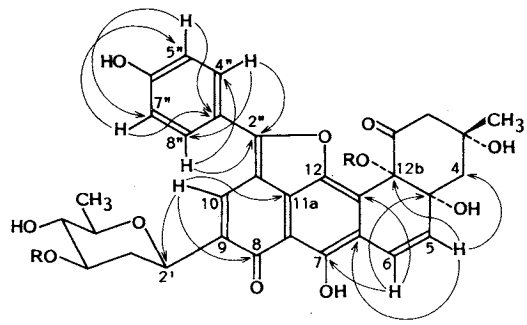
Table 1. ^{13}C NMR data for urdamycin H in comparison with those of urdamycin C (50.3 MHz, acetone-d_6, δ in ppm relative to internal TMS; multiplicities from DEPT analysis).[a]

	Urdamycin H (1)	Urdamycin C (2)		Urdamycin H (1)	Urdamycin C (2)
C-1	206.5 s	203.8 s	C-4″	130.5 d	123.7 s
C-2	54.8 t	54.6 t	C-5″	117.6 d	134.5 d
C-3	74.8 s	74.9 s	C-6″	162.0 s	115.9 d
3-CH_3	30.0 q	29.4 q	C-7″	117.6 d	160.0 s
C-4	43.9 t	44.0 t	C-8″	130.5 d	115.9 d
C-4a	81.7 s	82.2 s	C-9″		134.5 d
C-5	134.6 d	138.8 d	Sugar A		
C-6	119.8 d	118.6 d	C-1A	95.9 d	95.8 d
C-6a	117.7 s	125.2 s	C-2A	25.5 t	25.5 t
C-7	154.8 s	156.6 s	C-3A	25.1 t	25.1 t
C-7a	112.2 s	112.6 s	C-4A	76.4 d	76.4 d
C-8	184.8 s	187.5 s	C-5A	67.4 d	67.4 d
C-9	142.2 s	145.0 s	C-6A	17.3 q	17.4 q
C-10	128.9 d	134.7 d	Sugar B		
C-11	109.3 s	133.9 s	C-1B	102.7 d	102.3 d
C-11a	130.3 s	116.2 s	C-2B	40.3 t	40.3 t
C-12	144.0 s	143.7 s	C-3B	77.1 d	76.9 d
C-12a	123.7 s	128.9 s	C-4B	78.2 d	78.3 d
C-12b	83.7 s	83.1 s	C-5B	72.5 d	72.5 d
C-2′	72.3 d	72.1 d	C-6B	18.3 q	18.4 q
C-3′	38.1 t	37.7 t	Sugar C		
C-4′	79.7 d	79.2 d	C-1C	95.8 d	95.8 d
C-5′	76.4 d	76.4 d	C-2C	26.3 t	26.3 t
C-6′	72.0 d	71.8 d	C-3C	24.1 t	24.2 t
C-7′	18.8 q	18.6 q	C-4C	66.9 d	67.0 d
C-2″	161.0 s	159.5 s	C-5C	67.4 d	67.5 d
C-3″	121.3 s	132.7 s	C-6C	16.7 q	16.9 q

[a] These assignments (also the ones for the ^1H NMR data, see Experimental section) are confirmed by different NMR experiments. Previous assignments[8,9] for urdamycin C and other urdamycins have some slight errors (especially in the *C*-glycosidic and sugar moieties) and should be revised in analogy to the assignments presented here.

characterized by an enlarged aglycone (and a different chromophore) as in urdamycins C and D but without the aromatic δ-lactone carbonyl group, and with a *p*-substituted phenyl residue, as in urdamycin C. Everything else (the angucycline ring-system, the sugar moieties) is the same as in most of the other urdamycins. Thus structure **1** combines all of the characteristics; a furan moiety replaces the aromatic δ-lactone moiety of urdamycin C. The structure was supported by $^3J_{C\text{-}H}/^2J_{C\text{-}H}$ coupling experiments, *e.g.*, 2D hetero multiple bond connectivity spectroscopy (HMBC) and correlation spectroscopy *via* long range couplings (COLOC) NMR spectra, the results of which are summarized in Fig. 2.

Fig. 2. $^3J_{C\text{-}H}$ couplings of urdamycin H, detected *via* HMBC and/or COLOC.



$^2J_{C\text{-}H}$ couplings and the couplings in ring A and the *C*-glycosidic moiety are omitted for reasons of clarity.

Biosynthetic Studies

Since the biogenetic origin of the angucycline four-ring system, of the *C*-glycosidic moiety and of

the sugars has been investigated in urdamycins A~D,[10] and because it is very probable that urdamycin A, which is the biosynthetic precursor of the urdamycins C and D, is also the precursor of urdamycin H, the biosynthetic investigations were limited to the (*p*-OH-phenyl)furan moiety.

Feeding of [3,5-$^2H_2$]Tyrosine

To find out if the amino acid tyrosine is the precursor of the *p*-OH-phenyl residue (as it is in urdamycin C), the following experiment was carried out. Feeding 270 mg of [3,5-$^2H_2$]tyrosine to 2 liters fermentation broth in four portions (pulse feeding method) between 47 hours and 80 hours after inoculation resulted in the production of 15 mg urdamycin H (**1**), of which 30% was labeled by deuterium in the 5″/7″-positions (calculated from the integral of the 5″/7″ protons in the ^1H NMR spectrum). The labeling position was evident from the ^1H NMR spectrum of the deuterated urdamycin H showing the 4″/8″ protons (δ 8.00) as superimposed doublet ($J=8$ Hz) and singlet instead of the simple doublet ($J=8$ Hz) in the spectrum of nonlabeled urdamycin H.

Feeding of [3,5-$^2H_2$]*p*-OH-Phenylglycine

This experiment was done to determine if *p*-OH-phenylglycine, the homologue of tyrosine with one less carbon, is the immediate precursor of the (*p*-OH-phenyl)furan moiety of urdamycin H. This glycine derivative was assumed to be biotransformed from tyrosine by *S. fradiae* (Tü 2717), as is reported for the biogenetic origin of the *p*-OH-phenylglycine moiety in nocardicin A.[11] The [3,5-$^2H_2$]*p*-OH-phenylglycine was prepared from *p*-OH-phenylglycine by treatment with DCl-D_2O at 100°C using K_2PtCl_4 as a catalyst.[12,13] The product was 98% labeled exclusively at the 3,5-positions, yield >95%. The [3,5-$^2H_2$]*p*-OH-phenylglycine was fed at 250 mg/liter of culture as described above for [3,5-$^2H_2$]tyrosine, but the isolated urdamycin H (7 mg) did not contain significant deuterium.

Discussion

The structure of urdamycin H (**1**) demonstrates again the ability of the producing microorganism *S. fradiae* (Tü 2717) to create an unusual amount of aglycone and chromophore variation. Neither the aglycone of **1** nor its chromophore has been previously found in a natural product.

The attentive reader will not have failed to notice that one $^3J_{C\text{-}H}$ long range coupling of urdamycin H, namely the one from 10-H to C-2″ is missing in Fig. 2. This long range coupling was not observed because it is probably too small to be detectable. The difference between urdamycin H and urdamycin C or D, where one can see the analogous long range coupling, is (i) the less electron rich C-C bond (C-11–C-2″) because of the directly bonded oxygen, and (ii) the larger angle between the two C-C bonds (in urdamycins C and D: θ *ca.* 120°, in urdamycin H: θ *ca.* 135°, molecular model studies). Both factors cause a tremendous decrease in the $^3J_{C\text{-}H}$ coupling constant.[14,15]

The structure of urdamycin H (**1**) does not help to elucidate the biomechanistic question of the aglycone enlargement of urdamycin A into urdamycins C and D (and now also H). Obviously, urdamycin H cannot be an intermediate between urdamycins A and C; from the biosynthetic experiments it could rather be a subsequent product, caused by decarbonylation and ring contraction of the aromatic δ-lactone moiety of urdamycin C (**2**).

Experimental

General

See ref 1.

Isolation of Urdamycin H

The crude product of the Tü 2717 from 1-liter fermentation (see ref 10) was chromatographed on silica gel (column 30 × 3.5 cm, $CHCl_3$ - MeOH, 95 : 5). The orange urdamycin H fraction is covered by the blue urdamycin D; both components were separated by chromatography on silica gel (column, 2.5 × 20 cm, CH_2Cl_2 - MeOH, 85 : 15). A final purification on Sephadex LH-20 (column 2.5 × 100 cm, MeOH) gave 7 mg of urdamycin H as a dark orange solid powder: Rf 0.515 ($CHCl_3$ - MeOH, 4 : 1), 0.16 (CH_2Cl_2 - EtOH, 9 : 1); FAB-MS positive *m/z* 949 ($(M+H)^+$, 2%); negative *m/z* 947 ($(M-H)^-$, 4%); IR (KBr) cm^{-1} 3440, 2980, 2940, 2882 (sh), 1730, 1700 (sh), 1670, 1640, 1607, 1555, 1505, 1450, 1410 (sh), 1380, 1345, 1253, 1190, 1175, 1120, 1065, 980, 910, 850; ^1H NMR (200 MHz, acetone-d_6) δ 0.42 (d, J=6.5 Hz, 6C-H_3), 1.4 ~ 2.2 complex (3′-H_{ax}, 2B-H_{ax}, 2C-H_2, 2A-H_2, 3C-H_2, 3A-H_2), 1.16 (d, J=6.5 Hz, 6A-H_3), 1.14 (s, 3-CH_3), 1.22 (d, J=6 Hz, 6B-H_3), 1.38 (d, J=6 Hz, 7′-H_3), 1.80 ~ 2.35 complex (4-H_2), 2.17 (ddd obscured, J=13, 5 and 2 Hz, 2B-H_{ex}), 2.55 (ddd obscured, J=13, 5 and 2 Hz, 3′-H_{ex}), 2.76 and 3.25 (dd and d, J=13, 2 and 13 Hz, 2-H_2), 2.90 (dd, J=9 and 9 Hz, 4B-H), 3.16 (dd, J=9 and 9 Hz, 5′-H), 3.20 (dq, J=9 and 6 Hz, 5B-H), 3.29 (br s, 4C-H), 3.48 (ddd, J=13, 9 and 5 Hz, 3B-H), 3.52 (dq obscured, J=9 and 6 Hz, 6′-H), 3.55 (br s, 4A-H), 3.77 (dq, J=6.5 and 2 Hz, 5C-H), 3.77 (m obscured, 4′-H), 4.20 (dq, J=6.5 and 2 Hz, 5A-H), 4.60 (dd, J=10 and 2 Hz, 1B-H), 4.81 (dd, J=10 and 1.5 Hz, 2′-H), 5.00 (d, J=1.5 Hz, 1A-H), 5.24 (d, J=1.5 Hz, 1C-H), 5.98 (d, J=10 Hz, 5-H), 6.98 (d, J=10 Hz, 6-H), 7.15 (2H, d, J=8 Hz, 5″-H, 7″-H), 8.00 (2H, d, J=8 Hz, 4″-H, 8″-H), 8.38 (d, J=2 Hz, 10-H); UV λ_{max}^{MeOH} nm (ε) 240 (16,500), 265 (sh, 10,300), 304 (sh, 12,400), 326 (13,200), 335 (sh, 13,000), 388 (11,600), 402 (11,900), 424 (sh, 9,200), 490 (14,800); $\lambda_{max}^{MeOH-HCl}$ nm (ε) 238 (16,800), 260 (sh, 9,100), 302 (14,000), 320 (sh, 13,500), 386 (10,800), 405 (10,800), 486 (16,800); $\lambda_{max}^{MeOH-NaOH}$ 233 (sh, 15,100), 329 (10,300), 378 (11,300), 424 (14,000), 436 (17,300), 563 (17,800); CD $\lambda_{extreme}^{MeOH}$ nm $[\theta]^{22}$ 408 (−3,000), 375 (+2,000), 320 (−17,000), 295 (sh, −11,000), 268 (+11,000), 243 (−6,000), 228 (+6,000); ^{13}C NMR (50.3 MHz, acetone-d_6) see Table 1.

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