METABOLIC PRODUCTS OF MICROORGANISMS. 234[1] URDAMYCINS, NEW ANGUCYCLINE ANTIBIOTICS FROM *STREPTOMYCES FRADIAE*

I. ISOLATION, CHARACTERIZATION AND BIOLOGICAL PROPERTIES

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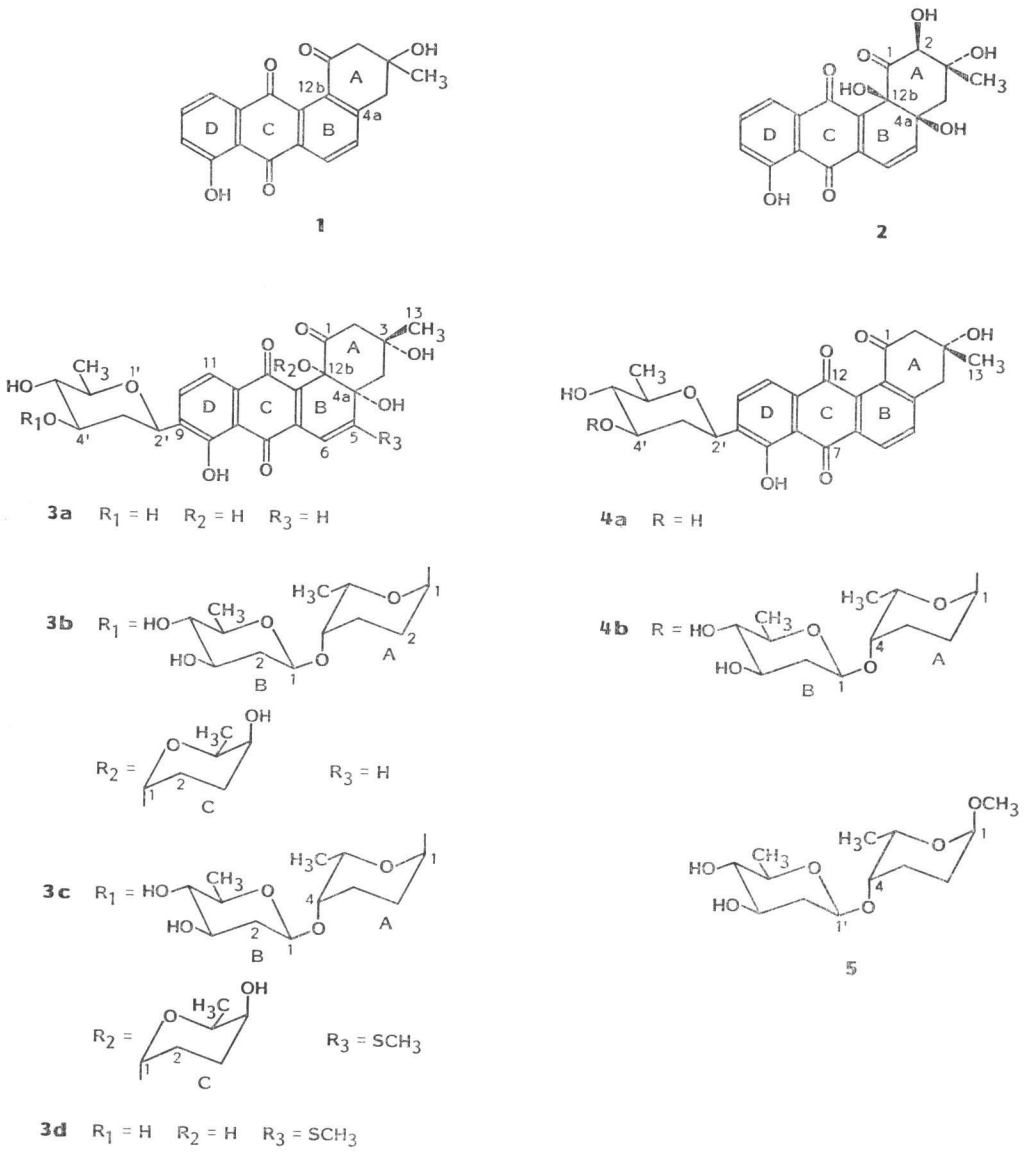
The colored urdamycins A to F, six new angucycline antibiotics produced by *Streptomyces fradiae* strain Tü 2717, were detected by chemical screening. They are biologically active against Gram-positive bacteria and stem cells of murine L1210 leukemia. The urdamycins are glycosides and differ in their aglycones, which can be liberated by acidic hydrolysis besides the sugars D-olivose and L-rhodinose. The structure of the main compound, urdamycin A (**3b**), follows from the spectroscopic and chemical data in connection with an X-ray analysis. The aglycone urdamycinone A (**3a**) is identical with aquayamycin. The structures of urdamycin B (**4b**), E (**3c**), F and partial structures of urdamycin C and D, will be presented in a subsequent paper. The new term "angucycline/angucyclinone" is used for an increasing group of related antibiotics.

It was shown recently that antibiotics containing a modified benz[a]anthraquinone as chromophoric systems are widespread. These antibiotics are reported to be active against bacteria and/or as enzyme inhibitors or antitumor agents. Until now these antibiotics had no characteristic group name, and we propose the new term "angucyclines/angucyclinones". This should be referenced to the angle (Latin: angus), which is a characteristic feature of the structures and biosynthesis as well, and to tetrangomycin (**1**), the compound with simplest and longest known structure among these antibiotics[2]. The proposed classification of a still growing group of antibiotics is advantageous because of the possibility to differentiate *O*-glycosides (angucycline) from those without any *O*-glycosidic linkage (angucyclinone), following the system of the well known anthracyclines.

Rings A and B in **1** could be modified by *cis*-orientated angular hydroxy groups at C-atoms 4a and 12b, as shown in sakyomicin B (**2**)[3] or, with the opposite stereochemistry, in aquayamycin (**3a**)[4,5]. Furthermore a β-olivoside residue is attached to the chromophoric system of **3a** by a *C*-glycosidic linkage. Among the angucyclinones the corresponding *C*-glycoside of tetrangomycin (**1**) was still missing; we shall describe it as aglycone **4a** of urdamycin B. Aquayamycin (**3a**) is the corresponding angucyclinone of several angucyclines, with sugars linked alternatively at 3-OH, 12b-OH, 4′-OH and/or 5′-OH, *e.g.* vineomycin A_1 (=P 1894 B)[5,6], the sakyomicins A and C[3], the saquayamycins[7] and the kerriamycins[8,9]. The recently published capoamycin[10] could be described as 5′-*O*-acyl angucyclinone. In the following we describe six new angucycline antibiotics, called urdamycin A to F.

Scheme 1.



The producing microorganism *Streptomyces fradiae* (strain Tü 2717) was isolated from a soil sample collected in Tanzania (Africa). The urdamycins were detected by chemical screening[11,12] because of their striking colors in TLC experiments (Table 1). Unlike other series of angucyclines the urdamycins differ in the aglycone part, whereas the sugar moieties are always the same. This is one characteristic of our strain. Furthermore it is a good example of the ability of microorganisms to produce secondary metabolites of related chemical structures in different proportions depending on the culture conditions (Table 1).

Fermentation and Isolation

Production of urdamycins was conducted in 500-ml Erlenmeyer flasks and in fermentors. Urda-

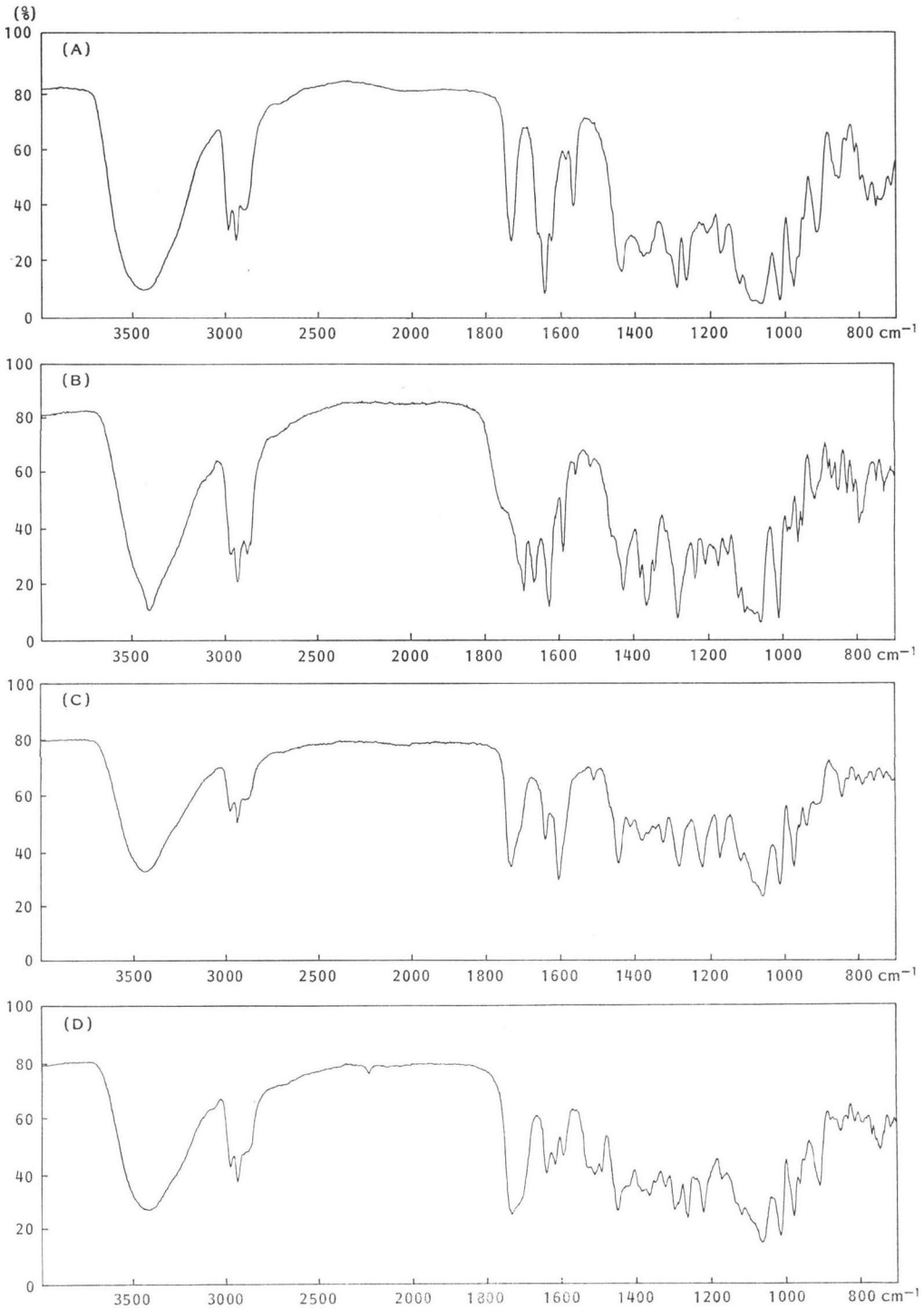
Table 1. Yields and physico-chemical data of the urdamycins.

Urdamycin	Molecular formula	MW	UV data and color, λ_{max} in nm (ε)		Rf value[a]		Yield in mg/liter (%)[b]	
			MeOH	MeOH - NaOH	I	II	A	B
A (**3b**)	$C_{43}H_{56}O_{17}$	844.9	440 (sh), 426 (5,500), 319 (4,500) Orange	580 (5,400), 404 (1,700), 325 (8,100) Ultramarine blue	0.55 (0.59)	0.24 (0.40)	38 (40)	5 (12)
B (**4b**)	$C_{37}H_{44}O_{13}$	696.8	403 (3,900), 268 (27,200) Dark yellow	505 (3,600), 315 (sh), 266 (17,300), 248 (14,100) Dark red	0.60 (0.60)	0.31 (0.39)	2 (2.5)	1 (2)
C	$C_{51\sim52}H_{58\sim62}O_{18\sim19}$	~980	510 (12,600), 410 (sh), 308 (14,800), 237 (sh), 226 (25,300) Dark red	625 (sh), 600 (12,100), 414 (5,500), 326 (12,000), 238 (30,000) Blue	0.51 (0.52)	0.15 (0.25)	6 (6)	5 (11)
D	$C_{53\sim55}H_{60\sim63}NO_{16\sim18}$	~1,000	575 (11,200), 328 (11,600) Violet	635 (sh), 600 (8,500), 412 (4,200), 324 (7,500), 270 (sh), 222 (32,400) Blue	0.52 (0.53)	0.17 (0.30)	1 (1)	1.3 (3)
E (**3c**)	$C_{44}H_{58}O_{17}S$	891.0	460 (4,500), 310 (6,300) Red	480 (5,200), 315 (5,000), 225 (16,300) Black	0.58 (0.61)	0.29 (0.45)	0.5 (0.5)	1 (1)
F	$C_{43}H_{58}O_{18}$	862.9	460 (sh), 432 (4,300), 278 (5,900), 250 (7,700), 218 (30,900) Orange	565 (4,500), 277 (6,600), 226 (25,900) Violet	0.44	0.11	0.01	—

[a] TLC silica gel, I: $CHCl_3$ - MeOH (4 : 1), II: CH_2Cl_2 - EtOH (9 : 1), in brackets Rf values of the corresponding aglycones.

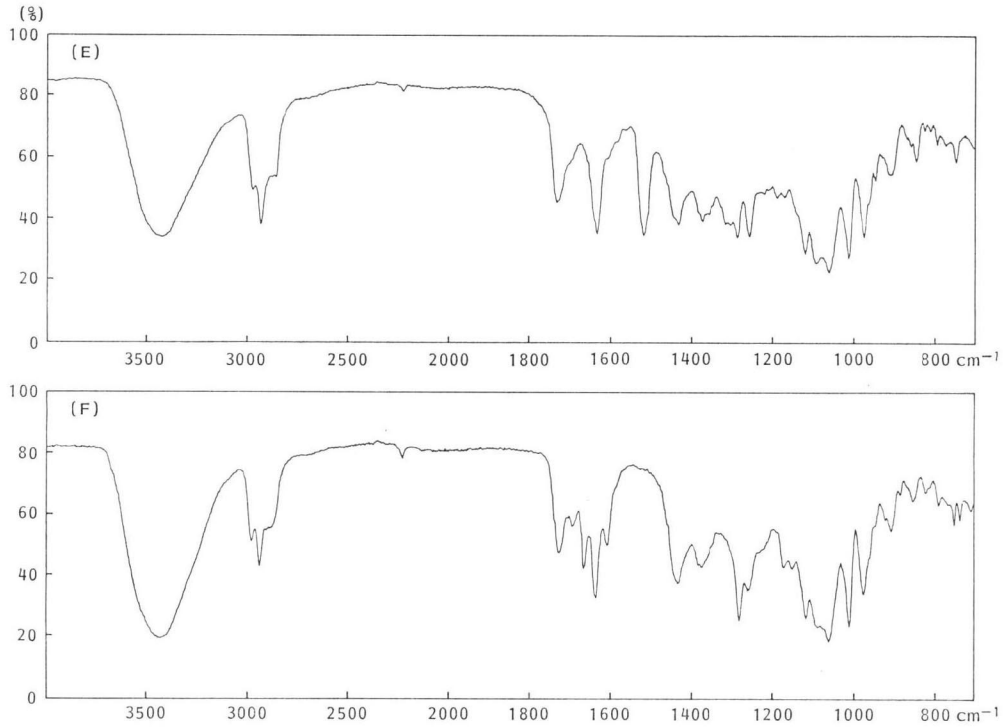
[b] Culture mediums A and B (see Experimental), % referred to raw product.

Fig. 1. IR spectra of urdamycins A to F in KBr.



mycin production was maximal at 72 hours, when the cultures were harvested. Depending on the culture conditions it is possible to enrich the dark colored urdamycins (C, D and E) relative to their light congeners (A, B and F). Table 1 shows the proportionate yields of the urdamycins in crude

Fig. 1. (Continued)



products, obtained under different culture conditions from two 100-liter fermentations.

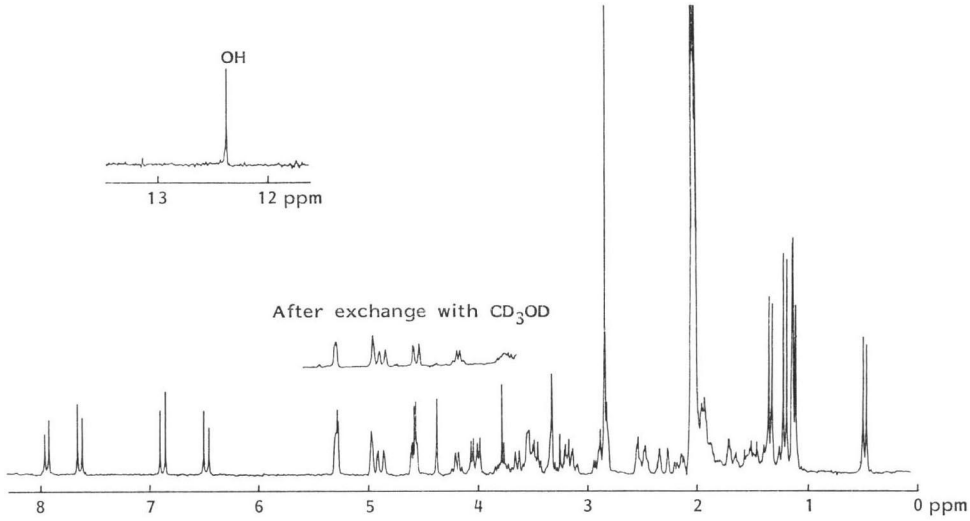
The crude product of strain Tü 2717, which was obtained by extraction from the mycelium (acetone) and the culture filtrate (ethyl acetate), was chromatographed in 5 g portions on silica gel with *chloroform* - methanol (4 : 1) as eluant. The five main fractions (and some mixed-fractions) yielded urdamycins B, E, A (main product), D and C. Urdamycin F was isolated from a subsequent orange zone, collected and pooled from 100-liter fermentations. A second chromatography on silica gel in chloroform - methanol or methylene chloride - ethanol systems (Rf values see Table 1) followed by a final purification on Sephadex LH-20 in methanol produced the pure compounds. The alphabetical order of the urdamycins results historically from the order of their detection.

The urdamycins were obtained as amorphous, hygroscopic powders by precipitation of their concentrated acetone solutions into *n*-pentane or *n*-hexane. They are soluble in DMSO, dioxane, acetone or alcohols and insoluble in water or alkanes. They are optically active (shown by CD spectra) and may be reduced to colorless hydroquinones by sodium dithionite.

Characterization and Structure of Urdamycin A (3b)

Urdamycin A, the main component of the urdamycin-complex, is an orange indicator with a change of the color to ultramarine blue at pH 7.7. Its 3-vinyl-5-hydroxy-1.4-naphthoquinone chromophore could be detected by typical UV (Table 1) and IR (Fig. 1) absorption bands. The NMR spectra and the elemental analysis were in agreement with the molecular formula $C_{43}H_{58}O_{17}$, which was confirmed by fast atom bombardment (FAB) mass spectroscopy. The complete molecule could be seen only as negative ion. This result was important to the structure elucidation of the other urdamycins. The mass spectrum did not show the expected $(M-H)^-$ ion at *m/z* 843, but a $(M+H)^-$ ion

Fig. 2. ^{1}H NMR spectrum of urdamycin A (**3b**) in acetone-d_6.



at m/z 845. In the mass spectrometer the molecule is reduced to the hydroquinone (M+2H), which looses one proton.

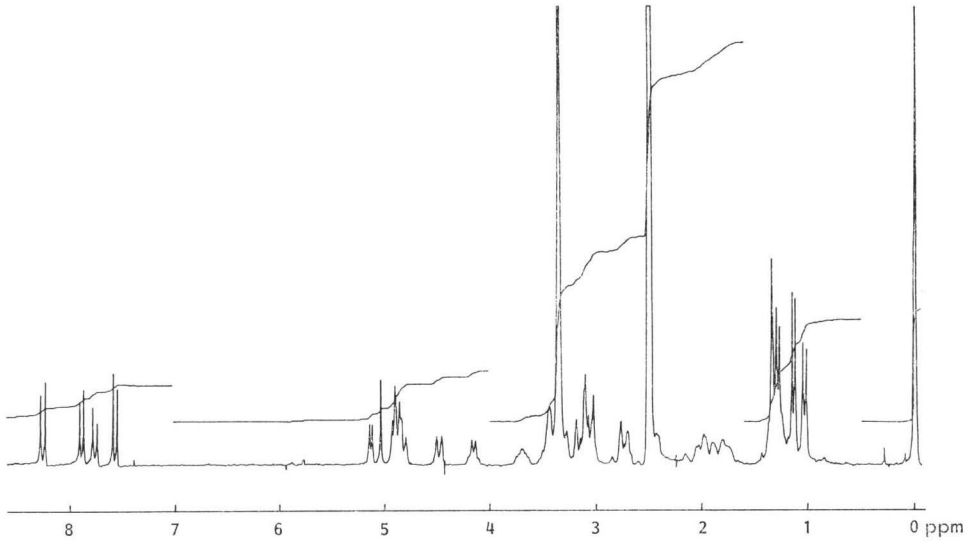
The NMR spectra indicated the presence of four 6-deoxyhexoses (three of them *O*-glycosidically - one *C*-glycosidically at the aromatic ring bonded). Especially diagnostic were four methyl doublets (δ 0.5~1.4) and four protons in the typical anomeric region (δ 4.6~5.4) of the ^{1}H NMR (Fig. 2) spectrum, but only three signals in the region of anomeric carbons (δ 91.8, 93.0 and 100.9) of the ^{13}C NMR spectrum. In the chromophore and *C*-glycosidic part urdamycin A (**3b**) could be related with aquayamycin[4~6] and differs from other aquayamycin-containing angucyclines in the nature and/or number of its sugar units. This conclusion was confirmed by the acidic hydrolysis, which resulted in the aglycone urdamycinone A, whose spectroscopic analysis and direct comparison with authentic material proved its identity with aquayamycin (**3a**). The disaccharide methyl-β-olivosyl-(1→4)-rhodinoside (**5**), which was obtained as the main sugar component by the acidic methanolysis of urdamycin A (**3b**), in connection with the NMR analysis demonstrates a second rhodinose to be the remaining sugar part. The upfield shift of one of the methyl doublets (δ 0.51) could be explained by the presence of a rhodinose, that is bonded angularly at C-12b (anisotropic effect of 1-CO), as it was observed in sakyomicin A and C[3].

The ^{1}H NMR analysis of the urdamycin A-pentaacetate (**3b**, but the four secondary and the phenolic OH acetylated) resulted in the ambiguity that the disaccharide moiety is bonded at C-4′ and the remaining rhodinose at C-12b or *vice versa*. This uncertainty and the absolute configuration of the *O*-glycosidically bonded sugars were solved by X-ray analysis of urdamycin A[13]. Thus the olivose has the β-D-, both rhodinoses the α-L-configuration, as seen in formula **3b**.

Characterization of Urdamycins B to F

The dark yellow urdamycin B (**4b**), the smallest molecule of the urdamycin complex, shows UV data (Table 1) in agreement with a 1-hydroxy-5,10-anthraquinone chromophore, the IR absorption band at 1705 cm^{-1} (Fig. 1) indicates an, α, β unsaturated ketone. The NMR spectra (Fig. 3) compared with those of **3b** show ring B to be aromatic and the lack of the angularly bonded rhodinose. Thus

Fig. 3. ^{1}H NMR spectrum of urdamycin B (**4b**) in DMSO-d_6.



urdamycin B (**4b**) is related to tetrangomycin[2], rabelomycin[14], ochromycinone[15] and the fujianmycins[16], which are angucyclinones with an anthraquinone chromophore. The molecular formula $C_{37}H_{44}O_{13}$ is supported by the NMR spectra and the high resolution EI(electron impact) mass spectrum (*m/z* 678, M$-H_2O$).

The dark red urdamycin C and the blue urdamycin D differ from the other urdamycins because they both posses larger chromophores (see UV data in Table 1) with unknown structure elements, correspondingly the IR spectra (Fig. 1) are quite different from those of **3b** or **4b**. Comparison of the NMR data with those of the other urdamycins and acidic hydrolysis prove the same sugar pattern for urdamycins C and D and additionally identical structural elements of the aglycones, namely the *C*-glycosidic bonded olivose and the complete A-ring could be seen.

The elemental analysis of urdamycin C results in 63.4% C and 6.7% H, no other elements, except oxygen, could be detected. In the NMR spectra about 51 C-atoms and about 60 H-atoms arise. From the FAB mass spectrum (*m/z* 978, negative ion) a molecular formula of $C_{51\sim52}H_{58\sim62}O_{18\sim19}$ was deduced. The field desorption (FD) mass spectrum results an ion at *m/z* 982. The comparison of the NMR spectra of urdamycin C with those of urdamycin A (**3b**) shows an evident difference in the aromatic/olefinic pattern: The olefinic AB system is shifted (δ 6.20/7.06, J=10 Hz), and the aromatic pattern is quite different (two equal AB systems, δ 7.06/7.47, J=8 Hz; one aromatic doublet, δ 7.96, J=2 Hz).

The elemental analysis of urdamycin D yielded 64.1% C, 6.5% H and 1.5% N besides oxygen. Thus urdamycin D is the only nitrogen containing compound of the urdamycin complex. The NMR analysis results about 53 C-atoms and 65 H-atoms; the molecular weight is about 1,000 (FD-MS: *m/z* 1,000, negative ion(neg)-FAB-MS: *m/z* 1,001). Thus the probable molecular formula is $C_{53\sim55}H_{60\sim68}NO_{16\sim18}$.

The rare urdamycin E (**3c**) demonstrates striking indicator properties: The blood red color in acidic and neutral solution changes to black, when it is treated with alkali (Table 1). There is a broad UV absorption band in alkaline solution (λ 400~750 nm), covering the whole visible region. In comparison with urdamycin A (**3b**) the shifts of the UV maxima indicate a hetero-atom in conjugation

Table 2. Antibacterial and antifungal assays of urdamycins A to E (disc-diffusion assay, inhibition diameter in mm).

Test microorganism	Urdamycin				
	A (3b)	B (4b)	C	D	E (3c)
Botrytis	—	—	—	—	—
Mucor 284	—	—	—	—	—
Paecilomyces	—	—	—	—	—
Bacillus subtilis^{+}	16	11	11	10	10
B. subtilis^{++}	24	11	11	10	17
B. brevis	15	10	8	9	14
Clostridium pasteurianum	13	11	—	10	13
Achromobacter geminianii	13	sp	—	—	—
Arthrobacter aurescens	—/13	8	—	—	sp
A. crystallopoides	20	13	sp	sp	11
Brevibacterium flavum	12	sp	—	sp	9
Corynebacterium rathayi	13/18	—	—	—	11
Saccharomyces cerevisiae	—	—	—	—	—
S. phaeochromogenes (Tü 4)	10	—	—	—	11
Streptomyces griseus (Tü 17)	12	—	—	—	—/11
S. diastatochromogenes (Tü 20)	10	—	—	—	15
S. violaceoruber (Tü 22)	12/18	sp	—	—	10
S. prasinus (Tü 30)	18	16	—	—	8
S. lavendulae (Tü 35)	22	12	—	10	11
S. glaucescens (Tü 49)	20	sp	—	—	14
S. viridochromogenes (Tü 57)	24	10	10	11	18
S. violaceus-niger (Tü 1418)	12	—	—	—	10

sp: Trace, —: no inhibition, concentration: 1 mg/ml each, double numbers: first complete inhibition, second partial inhibition, +: complex media, ++: chemically defined media.

Table 3. Anticancer assays against L1210 leukemia cells (IC_{50} values in μg/ml)[15].

	Proliferation assay	Stem cell assay
Urdamycin A (**3b**)	2.4	0.55
Aquayamycin (**3a**)	2.8	0.38
Urdamycin B (**4b**)	10.0	1.90
Urdamycinone B (**4a**)	3.1	3.40
Urdamycin C	10.0	6.60
Urdamycinone C	10.0	2.20
Urdamycin D	10.0	5.70
Urdamycin E (**3c**)	1.7	0.47
Adriamycin	0.02	0.022

with the chromophore. The molecular formula $C_{44}H_{58}O_{17}S$ is in agreement with the elemental analysis and the FAB mass spectrum giving an $(M+1)^-$ ion (*m/z* 891) analogous to **3b**.

The orange urdamycin F is the rarest component of the urdamycin-complex (see Table 1), and also the most hydrophilic one. The neg-FAB mass spectrum (*m/z* 862), the elemental analysis and the NMR spectra fit with the molecular formula $C_{43}H_{55}O_{18}$ and show that one molecule of water is added to urdamycin A.

Biological Activities

In the disc-diffusion assay the sensitivity of various bacteria and fungi were tested against the urdamycins. The results show that the biological activity spectrum of the urdamycins includes Gram-positive and Gram-negative bacteria but no fungi (Table 2). The high antibacterial activity against streptomycetes is reminiscent of tetracenomycin C[17] and elloramycin[12]. The results of the stem cell assay against L1210 leukemia cells and the proliferation assay in comparison with adriamycin (doxorubicin) are shown also in Table 3[15]. For both tested activities the most active compounds are urdamycin A (**3b**) and urdamycin E (**3c**). The *in vivo* test of **3b** against P388 leukemia show no

positive T/C values and toxicity from 55 mg/kg on.

Discussion

The urdamycins are new angucyclines. They differ from other series of *O*-glycosides because of their variety of aglycones, while the sugar moieties are always the same. This fact results in the widespread spectrum of colors to be able observable while working with urdamycins. Structure elucidation of urdamycin in B to F will be described in a following paper. Urdamycin E (**3c**) is the first sulfur-containing angucycline. Urdamycin A (**3b**) is probably identical with one of the recently published angucyclines, namely with kerriamycin B, but the given formula[8,9] shows a different sugar moiety (L-olivose) as described in the text (D-olivose). The structure elucidation of urdamycin A independently followed from chemical derivatization and an X-ray analysis[13], which also confirms the results.

Except urdamycin B (**4b**), the urdamycins contain sugar moieties on both sides of the aglycone. In this they resemble some aureolic acid antibiotics[19,20]. Thus the mode of action in biologic activities is probably quite different from the anthracyclines, *e.g.* no intercalation could be observed while testing the anticancer activity[18]. Another difference between the anthracyclines and the angucyclines is the lack of amino sugars in the latter group until now.

Experimental

General

Melting points were determined using a Reichert hot stage microscope. UV spectra were recorded using a Zeiss DMR 21 spectrometer, IR spectra were obtained in pressed KBr discs using a Perkin-Elmer Model 298 spectrometer. The NMR spectra were determined with a Varian FT 80 (1.9 T), XL-100 (2.3 T) or XL-200 (4.7 T), respectively. Chemical shifts (δ in ppm) are reported relative to internal tetramethylsilane; the full data will be given in a following paper. The mass spectra were obtained on a Varian MAT 731 or a Varian 311 A, respectively, using direct probe insert, high resolutions with perfluorokerosine as a standard. The FAB mass spectra were obtained on a Finnigan MAT 8230, using glycerol or trihydroxybutane as the matrix. CD spectra were recorded using a Jasco J 500 A spectrometer in combination with a BMC if 800 personal computer. Thin-layer chromatography (TLC) was performed on silica gel plates (Macherey & Nagel Sil G/UV 254+366, 0.25 mm silica gel on glass), column chromatography on Silica gel 60 (<0.08 mm, Macherey & Nagel).

Bacterial Strains

The standard strains for the activity spectrum analysis of the urdamycins were obtained from the stock culture collection in our laboratories or from ATCC. The antibiotic producing microorganism (Tü 2717) was a new soil isolate from Tanzania (Africa), classified according to HÜTTER[21] and BERGEY[22] as *Streptomyces fradiae*.

Fermentation of the Urdamycins

Streptomyces fradiae was cultured for 72 hours at 27°C in medium I consisting of meat meal 2%, malt extract 10%, $CaCO_3$ 1% or medium II consisting malt extract 2%, the residue of ethanol production (distillers solubles) 2%, NaCl 0.5%, $NaNO_2$ 0.1% (100 ml in 500-ml Erlenmeyer flask). The pH was adjusted to 7.2 before autoclaving.

Biological Assay

The disc-diffusion assay was used for measuring the antibiotic content of the cultures, and to determine the antibacterial and antifungal spectra of the urdamycins and their derivatives.

Isolation of Urdamycins A to F

The mycelium was extracted with acetone, the culture filtrate with ethyl acetate, at pH 7. The pooled organic layers were dried and evaporated, the remaining syrup was precipitated by pouring into petroleum ether.

This crude product was chromatographed on silica gel (column 30×10.5 cm, $CHCl_3$-

EtOH, 4:1) to yield 6 fractions in the following sequence, chiefly enriched with the antibiotic in brackets: 1) Yellow (urdamycin B), 2) red (urdamycin E), 3) orange (urdamycin A), 4) blue (urdamycin D), 5) dark red (urdamycin C), 6) orange (urdamycin F).

Sometimes some of the aglycones (urdamycinone A, B, C and E) could be found in the raw product, probably as an artifact of work-up.

Urdamycin A (**3b**)

300 mg of the orange fraction 3) was purified by chromatography on silica gel (column 50×4 cm, CH_2Cl_2 - MeOH, 9:1) and Sephadex LH-20 (column 200×2.5 cm, MeOH), respectively, to yield 240 mg urdamycin A (**3b**). The antibiotic was precipitated by pouring its concentrated acetone solution into *n*-hexane: MP 160°C (dec); Rf values see Table 1; IR (KBr, Fig. 1) 3430, 1728, 1657 (sh), 1650, 1639, 1620 cm^{-1}; UV see Table 1; ^1H NMR see Fig. 2; neg-FAB-MS *m/z* 845 (M+2H − H); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{24}$) 456 (+5,000), 400 (−9,000), 328 (+35,000), 290 (sh, +11,000), 261 (−5,000), 232 (+38,000).

Anal Calcd for $C_{43}H_{56}O_{17}$: C 61.13, H 8.68.
Found: C 60.47, H 6.66.

Urdamycinone A (**3a** =Aquayamycin)

250 mg **3b** were stirred in a mixture of TFA - MeOH - H_2O (1:2:2) for 10 minutes at room temperature. The acid was removed by distribution of the solution between EtOAc and H_2O. The organic layer was evaporated to dryness, dissolved again in MeOH and chromatographed on Sephadex LH-20 (column 100×2.5 cm, MeOH), to yield 160 mg **3a**, which was identical with respect to all spectroscopic data with literature[4,5]: CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{24}$) 450 (+3,000), 395 (−4,000), 300 (sh, +13,000), 267 (+14,000), 247 (−2,000), 230 (sh, +16,000).

Methyl-2,6-dideoxy-β-D-*arabino*-hexopyranosyl-(1→4)-2,3,6-trideoxy-α-L-*threo*-hexopyranoside (**5**: Methyl-β-D-olivosyl-(1→4)-α-L-rhodinoside)

490 mg urdamycin A were dissolved in a mixture of 70 ml MeOH - H_2O (5:2) and treated with 20 drops of conc H_2SO_4. It was stirred for *ca.* 30 minutes till no more urdamycin A could be detected by TLC control. The solution was diluted with 200 ml of H_2O and neutralized with a saturated $Ba(OH)_2$ solution using a pH meter. The precipitate was removed by centrifugation, the solvents were evaporated to dryness. The residue was chromatographed several times on Sephadex LH-20 (column 200×2.5 cm, MeOH), to yield aquayamycin (**3a**) and the disaccharide **5** (the main sugar component) as colorless syrup (the sugars were detected by dipping the TLC-plates into a 7% molybdatophosphoric acid containing ethanol solution): Rf 0.59 ($CHCl_3$ - MeOH, 4:1), 0.36 (CH_2Cl_2 - EtOH, 9:1); ^1H NMR (80 MHz, CD_3OD) δ 1.12 (3H, d, J=6.5 Hz, 5-CH_3), 1.23 (3H, d, J=6 Hz, 5′-CH_3), *ca.* 1.25 (1H, ddd, J=13, 12, 11 Hz, 2′-H_{ax}), 1.4~2.0 (4H, complex, 2-H_2, 3-H_2), 2.16 (1H, ddd, J=13, 5, 2 Hz, 2′-H_{eq}), 2.85 (1H, dd, J=9.5, 9 Hz, 4′-H), *ca.* 3.2 (obscured, 5′-H), 3.31 (3H, s, 1-OCH_3), 3.45 (1H, ddd, J=12, 9, 5 Hz, 3′-H), 3.47 (1H, br s, 4-H), 3.83 (1H, dq, J=6.5, 2 Hz, 5-H), 4.52 (1H, dd, J=11, 2 Hz, 1′-H), 4.58 (1H, br s, 1-H); ^{13}C NMR (50.3 MHz, CD_3OD) δ 17.4 (q, C-6), 18.3 (q, C-6′), 25.1 (t, C-3), 25.6 (t, C-2), 40.6 (t, C-2′), 54.9 (q, 1-OCH_3), 67.4 (d, C-4), 72.3 (d, C-5), 73.2 (d, C-5′), 77.5 (d, C-3′), 78.4 (d, C-4′), 99.7 (d, C-1), 102.8 (d, C-1′).

Urdamycin A-pentaacetate

60 mg of urdamycin A (**3b**) were dissolved in a mixture of 10 ml acetic anhydride and 5 ml pyridine. After stirring for 7 hours at room temperature the solution was poured into ice-water. It was extracted 3 times with 50 ml $CHCl_3$ and evaporated to dryness. Pyridine residues were removed by dissolving several times in toluene and evaporating. The residue was chromatographed on silica gel (plates 20×40 cm, $CHCl_3$ - MeOH, 98:2), the yellow main zone was rechromatographed at Sephadex LH-20 (column 50×2.5 cm, MeOH), to yield 23 mg urdamycin A-pentaacetate: MP 185°C; Rf 0.83 ($CHCl_3$ - MeOH, 4:1), 0.91 (CH_2Cl_2 - EtOH, 9:1); IR (KBr) 3470, 1783, 1741, 1667, 1632, 1599 cm^{-1}; UV λ_{max} nm (ε) (MeOH and MeOH - HCl) 355 (4,300), 313 (4,900), 257 (15,900); (MeOH - NaOH) 395 (sh, 2,600), 375 (sh, 3,500), 327 (7,400), 253 (10,400); ^1H NMR (200 MHz, $CDCl_3$) δ 0.50 (3H, d, J=6.5 Hz, 5C-CH_3), 1.18 (3H, d, J=6.5 Hz, 5A-CH_3), 1.19 (3H, d, J=6 Hz, 5B-CH_3), 1.25

(3H, s, 3-CH_3), 1.27 (3H, d, J=6 Hz, 6'-CH_3), 1.2~2.2 (13H, complex), 2.00, 2.04, 2.06 and 2.10 (3H each, 4s, 5'-, 3B-, 4B- and 4C-OAc), 2.36 (1H, ddd, J=13, 5, 2 Hz, 2B-H_{eq}), 2.48 (3H, s, 8-OAc), 2.52 and 2.80 (1H, each, d and dd, J=13 and 13, 2 Hz, 2-H_2), 3.44 (1H, dq, J=9, 6 Hz, 5B-H), 3.48 (1H, br s, 4A-H), 3.57 (1H, s, exchange with CD_3OD, OH), *ca.* 3.6 (obscured, 6'-H), 3.68 (1H, dq, J=6.5, 2 Hz, 5C-H), 3.87 (1H, dq, J=6.5, 2 Hz, 5A-H), 4.04 (1H, m, 4'-H), 4.06 (1H, s, exchange with CD_3OD, OH), 4.56 (1H, dd, J=10, 2 Hz, 1B-H), 4.67 (1H, br s, 4C-H), 4.6~4.8 (obscured, 2'-H), 4.74 and 4.82 (1H each, dd and dd, J=9, 9 Hz each, 5'- and 4B-H), 4.90~5.02 (2H, complex, 1A- and 3B-H), 5.46 (1H, d, J=2 Hz, 1C-H), 6.41 (1H, d, J=10 Hz, 5-H), 6.85 (1H, d, J=10 Hz, 6-H), 8.02 (1H, d, J=8 Hz, 10-H), 8.13 (1H, d, J=8 Hz, 11-H); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{22}$) 340 (−14,000), 280 (+43,000), 250 (+32,000), 226 (+58,000).

Anal Calcd for $C_{53}H_{66}O_{22}$: C 60.33, H 6.31.
Found: C 60.41, H 6.40.

Urdamycin B (4b)

450 mg of the yellow fraction 1) were chromatographed again on silica gel (column 25×6 cm, $CHCl_3$ - MeOH, 9:1) and Sephadex LH-20 (column 200×2.5 cm, MeOH), to yield 370 mg of **4b** and 15 mg of urdamycin E. **4b** was precipitated by pouring its concentrated acetone solution into *n*-hexane: Rf see Table 1; IR (KBr, Fig. 1) 3400, 1750 (sh), 1705 (sh), 1694, 1668, 1626, 1589 cm^{-1}; UV (MeOH) see Table 1; ^1H NMR see Fig. 3; MS (70 eV) *m/z* (abundance) 678 (0.5%, M−H_2O, high resolution calcd for $C_{37}H_{42}O_{12}$ and found: 678.2676,) 548 (2%, 678−olivose, high resolution calcd for $C_{31}H_{32}O_9$ and found: 548.2046), 434 (55%, 548−rhodinose, high resolution calcd for $C_{25}H_{22}O_7$ and found: 434.1366), 330 (38%), 147 (22%), 131 (41%), 113 (34%), 96 (100%), 81 (91%), 69 (47%), 57 (51%), 43 (89%); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{20}$) 415 (+2,000), 375 (+300), 330 (+2,000), 271 (−18,000), 227 (+8,000).

Urdamycin C

400 mg of the dark red fraction 5) were purified chromatographically on silica gel (column 25× 6 cm, CH_2Cl_2 - EtOH, 9:1) and Sephadex LH-20 (column 200×2.5 cm, MeOH) and precipitated, when its concentrated acetone solution was poured into *n*-pentane, to yield 310 mg urdamycin C: MP 200°C (dec); Rf see Table 1; IR (KBr, Fig. 1) 3420, 1732, 1705, 1640, 1605 cm^{-1}; UV (MeOH) see Table 1; ^1H NMR (200 MHz, acetone-d_6) δ 0.39 (d, J=6.5 Hz, 5C-CH_3), 1.12 (s, 3-CH_3), 1.13 (d, J=6.5 Hz, 5A-CH_3), 1.22 (d, J=6 Hz, 5B-CH_3), 1.23 (d, J=6 Hz, 6'-CH_3), 1.2~1.6 (8~10H, complex), 1.8~2.2 (partly obscured, 4-H_2), 2.18 (ddd, J=13, 5, 2 Hz, 3'-H_{eq}), 2.58 (ddd, J=13, 5, 2 Hz, 2B-H_{eq}), 2.70 (dd, J=13, 2 Hz, 2-H_{eq}), 2.92 (dd, J=9, 9 Hz, 5'-H, observable after exchange with D_2O), 3.06 (ddd, J=9, 9, 4.5 Hz, after D_2O-exchange: dd, J=9, 9 Hz, 4B-H), 3.16 (d, J=13 Hz, 2-H_{ax}), 3.33 (s, 4C-H), *ca.* 3.3~3.4 (obscured, 5B-H), 3.48 (dq, J=9, 6 Hz, 6'-H), *ca.* 3.5 (obscured, 3B-H), 3.55 (s, 4A-H), 3.63 (dq, J=6.5, 2 Hz, 5C-H), 3.79 (ddd, J=12, 9, 5 Hz, 4'-H), 3.80 (s, OH, exchange with D_2O), 4.05 (d, J=4.5 Hz, OH, exchange with D_2O), 4.10 (d, J=4.5 Hz, OH, exchange with D_2O), 4.20 (dq, J=6.5, 2 Hz, 5A-H), 4.56 (s, OH, exchange with D_2O), 4.59 (d, J=3.5 Hz, OH, exchange with D_2O), 4.60 (dd, J=10, 1.5 Hz, 1B-H), 4.75 (dd, J=10, 1.5 Hz, 2'-H), 5.00 (s, 1A-H), 5.29 (s, OH, exchange with D_2O), 5.44 (s, 1C-H), 6.20 (d, 1H, J=10 Hz), 7.05 (d, 1H, J=10 Hz), 7.06 (d, 2H, J=8 Hz), 7.47 (d, 2H, J=8 Hz), 7.96 (d, 1H, J=2 Hz), 9.10 (s, OH, exchange with D_2O), 13.16 (s, OH, exchange with D_2O); neg-FAB-MS *m/z* 978 (100%); FD-MS *m/z* 982 (100%); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{21}$) 500 (−5,000), 336 (+17,000), 283 (−12,000), 248 (+29,000), 236 (+22,000), 223 (+48,000).

Anal Found: C 63.37, H 6.73.

Urdamycin D

240 mg of the blue fraction 4) were chromatographed again on silica gel (column 50×4 cm, CH_2Cl_2 - MeOH, 85:15) and Sephadex LH-20 (column 100×2.5 cm, MeOH), to yield 18 mg urdamycin A, 85 mg urdamycin D and 50 mg urdamycin C, respectively. Urdamycin D was precipitated as blue, amorphous powder by pouring its concentrated acetone solution into *n*-hexane: MP 220°C (dec); Rf see Table 1; IR (KBr, Fig. 1) 3420, 1735, 1710 (sh), 1690, 1668, 1595 cm^{-1}; UV (MeOH) see Table 1; ^1H NMR (200 MHz, acetone-d_6) δ 0.41 (d, J=6.5 Hz, 5C-CH_3), 1.07 (d, J=6 Hz, 5B-

CH_3), 1.13 (d, J=6.5 Hz, 5A-CH_3), 1.20 (s, 3-CH_3), 1.22 (d, J=6 Hz, 6'-CH_3), 1.2~1.6 (8~10H, complex), 1.8~2.2 (partly obscured, 4-H_2, 3'-H_{eq}), 2.58 (ddd, J=13, 5, 2 Hz, 2B-H_{eq}), 2.70 (dd, J=13, 2 Hz, 2-H_{eq}), 2.91 (dd, J=9, 9 Hz, 5'-H), 2.95 (dd, J=9, 9 Hz, 4B-H), 3.20 (d, J=13 Hz, 2-H_{ax}), 3.24 (dq, J=9, 6 Hz, 5B-H), 3.26 (s, 4C-H), 3.42 (dq, J=9, 6 Hz, 6'-H), 3.53 (m, 3B-H), 3.57 (s, 4A-H), 3.64 (dq, J=6.5, 1 Hz, 5C-H), 3.77 (ddd, J=12, 9, 5 Hz, 4'-H), 4.17 (dq, J=6.5, 1.5 Hz, 5A-H), 4.59 (dd, J=10, 2 Hz, 1B-H), 4.60 (s, OH, exchange with D_2O), 4.77 (dd, J=10, 1.5 Hz, 2'-H), 5.00 (d, J=1.5 Hz, 1A-H), 5.26 (s, OH, exchange with D_2O), 5.46 (d, J=1 Hz, 1C-H), 6.16 (d, 1H, J=10 Hz), 7.06 (d, 1H, J=10 Hz), 7.20 (dt, 1H, J=7.5, 1.5 Hz), 7.29 (dt, 1H, J=7.5, 1.5 Hz), 7.62 (dd, 1H, J=7.5, 1.5 Hz), 7.63 (dd, 1H, J=7.5, 1.5 Hz), 8.12 (s, 1H), 8.25 (d, 1H, J=1.5 Hz), 11.26 (s, OH, exchange with D_2O); neg-FAB-MS m/z 1,001 (88%); FD-MS m/z 1,000 (5%); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{24}$) 440 (+1,000), 395 (−3,000), 350 (+1,000), 320 (sh, −4,000), 278 (−15,000), 245 (+20,000), 226 (+59,000).

Anal Found: C 64.11, H 6.46, N 1.51.

Urdamycin E (**3c**)

190 mg of the red fraction 2) were chromatographed again on silica gel (column 50×3.5 cm, $CHCl_3$ - MeOH, 85:15) and Sephadex LH-20 (column 100×2.5 cm, MeOH), to yield 60 mg urdamycin B, 50 mg urdamycin E and 10 mg urdamycin A, respectively. Urdamycin E (**3c**) was precipitated as red amorphous powder by pouring its concentrated acetone solution into *n*-hexane: Rf see Table 1; IR (KBr, Fig. 1) 3430, 1733, 1700 (sh), 1635, 1610 (sh) cm^{-1}; UV (MeOH) see Table 1; neg-FAB-MS m/z 891 (29%); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{22}$) 495 (−2,000), 460 (+1,000), 420 (−4,000), 325 (+11,000), 253 (+29,000), 226 (+3,000).

Anal Calcd for $C_{44}H_{55}O_{17}S$: C 59.31, H 6.56, S 3.60.
Found: C 59.42, H 6.72, S 2.28.

Urdamycin F (**7**)

The collected later-running bands of different prechromatographies (several 100-liter fermentors) were purified chromatographically on silica gel (column 40×3 cm, $CHCl_3$ - MeOH, 9:1) and Sephadex LH-20 (column 50×2.5 cm, MeOH), to yield 20 mg pure urdamycin F (**7**): Rf see Table 1; IR (KBr, Fig. 1) 3440, 1727, 1694, 1666, 1635, 1608 cm^{-1}; UV (MeOH) see Table 1; neg-FAB-MS m/z 862 (7%); CD $\lambda_{extreme}$ (MeOH) nm ($[\theta]^{26}$) 495 (−3,000), 450 (+2,000), 350 (−11,000), 310 (sh, −1,000), 275 (+15,000), 243 (−3,000).

Anal Calcd for $C_{43}H_{58}O_{18}$: C 59.85, H 6.77.
Found: C 59.45, H 7.30.

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