Tetrahedron. Vol. 30, pp. 2747 to 2754. Pergamon Press 1974. Printed in Great Britain

STRUCTURE DETERMINATION OF PURPUROMYCIN, A NEW ANTIBIOTIC

M. R. BARDONE, E. MARTINELLI, L. F. ZERILLI and C. CORONELLI

Research Laboratories, Gruppo Lepetit S.p.A., via Durando, 38, 20158 Milano, Italy

(*Received in the UK 29 October* 1973; *Accepted for publication 25 February* 1974)

Abstract—From a culture broth of *Actinoplanes ianthinogenes* a red crystalline product was isolated displaying activity against bacteria and fungi. The structure of the new antibiotic, named purpuromycin, was established on the basis of chemico-physical evidences and chemical degradations. The product resulted to be structurally related to rubromycins, a group of antibiotics previously isolated from *Streptomyces collinus*.

Purpuromycin is a metabolite isolated from *Actinoplanes ianthinogenes* cultures provided with *in vitro* activity against bacteria and fungi.[1] The product obtained from the culture broth by extraction with ethyl acetate was purified by column chromatography on silica-gel and crystallised from chloroform-methanol.

Purpuromycin (**1**) appears as bright purple crystals (dec 212°). Elemental analysis and hydrogen number from ^{1}H NMR spectrum account for a molecular formula $C_{26}H_{18}O_{13}$ (M.W. 538·40). The uv and visible absorption maxima (Fig 1), the redox potential value ($E_0 = +0{\cdot}173$ volt)[2] and the easy reduction with a variety of reducing agents (zinc and dilute hydrochloric acid or sodium dithionate) suggest the presence of a naphthoquinone moiety. The lacking of quinoxaline derivative formation with *o*-phenylenediamine points to a 1,4 naphthoquinone. Formation of boroacetic ester[3] indicated by a bathochromic shift of the visible maxima, fluorescence in glacial acetic acid[4] and the shifting of the IR carbonyl absorption to higher frequency upon acetylation ($1600 \rightarrow 1650\ cm^{-1}$) are consistent with the presence of a naphthazarin moiety.

A comparison of the chemico-physical properties of purpuromycin with those of known antibiotics containing the naphthazarin moiety showed a close similarity with rubromycins, a group of antibiotics obtained from *Streptomyces collinus*.[5] In particular, the electronic spectrum of purpuromycin is identical to that of γ-rubromycin (**1a**)[6] which, however, contains one oxygen atom less and shows higher mobility in TLC analysis.* The mass spectra of the two compounds were also different: purpuromycin does not show the M^+ at *m/e* 538 (Table 1) whereas γ-rubromycin shows a relevant M^+ at *m/e* 522.[6] The highest mass fragment at *m/e* 520 present in the spectrum of purpuromycin was attributed to $[M-H_2O]^+$. This interpretation was confirmed by the mass spectrum of the peracetylderivative in which the M^+ at *m/e* 760 accounts for the introduction of four acetyl groups in a molecule having M.W. 538. The most significant peaks in the low mass range of the spectrum of purpuromycin are the couple at *m/e* 274, 273 present also in the spectrum of γ-rubromycin and attributed to the naphthazarin moiety.[6] Peaks at *m/e* 286 and 264, which are present only in the spectrum of purpuromycin, resulted characteristic of the naphthazarin and the isocoumarin moiety, respectively.[7] The ^{1}H NMR data of purpuromycin and of γ-rubromycin in $CDCl_3$ and in CF_3CO_2D are reported in Tables 2 and 3. Both compounds reveal the presence of three chelated phenolic hydroxyls, three aromatic/olefinic protons (one of which is at lower field in purpuromycin, δ 7·30 *vs* 6·95), two methoxyls and two non equivalent protons of a methylene group. Instead of the $-CH_2-CH_2-$ sequence of γ-rubromycin, purpuromycin shows signals corresponding to a $-CH_2-CH(OH)-$ moiety: the alcoholic hydroxyl is on the methine as indicated by the 1·4 ppm paramagnetic shift of the methine proton upon acetylation (δ 4·8 → 6·2) (Table 2). The IR spectrum of purpuromycin indicates the presence of the functionalities described in Table 4 which were assigned also on the basis of the IR spectra of the transformation products described afterwards.

On the basis of the chemico-physical evidences reported, structure **1** can be proposed for purpuromycin. The optical activity of **1** was not measured and therefore no considerations can be done on the chiral centers of purpuromycin.

Structure **1** was substantiated by chemical degra-

*Samples of rubromycins were kindly supplied by Prof. Brockmann whom we wish to thank.

Table 1. Selected mass spectral data of purpuromycin and related compounds

Compound	*m/e*, (relative intensity) and [attribution] of selected ions								
1	520 (62) $[M-H_2O]^{+\cdot}$	286[a] (41) $[M-C_{11}H_8O_7]^{+\cdot}$	274[a] (100) $[M-C_{12}H_8O_7]^{+\cdot}$	273 (19) $[M-\dot{C}_{12}H_9O_7]^{+}$	264[a] (24) $[M-C_{14}H_{10}O_6]^{+\cdot}$	256 (29) $[274-H_2O]^{+\cdot}$	250[a] (37) $[M-C_{14}H_8O_7]^{+\cdot}$	245 (32) $[274-\dot{C}HO]^{+}$	236[a] (86) $[264-CO]^{+\cdot}$[b]
Tetraacetyl I[c]	706 (1) $[M]^{+\cdot}$	646 (15) $[M-CH_3CO_2H]^{+\cdot}$	604 (8) $[646-COCH_2]^{+\cdot}$		562 (30) $[604-COCH_2]^{+\cdot}$	520 (100) $[562-COCH_2]^{+\cdot}$			
1a	522 (36) $[M]^{+\cdot}$	274 (100) $[M-C_{12}H_8O_6]^{+\cdot}$	273 (57) $[M-\dot{C}_{12}H_9O_6]^{+}$		256 (15) $[274-H_2O]^{+\cdot}$	250 (62) $[M-C_{14}H_8O_6]^{+\cdot}$	245 (22) $[274-\dot{C}HO]^{+}$	236 (8) $[M-C_{15}H_{10}O_6]^{+\cdot}$	
2	538 (3) $[M]^{+\cdot}$	520 (68) $[M-H_2O]^{+\cdot}$	274 (100) $[M-C_{12}H_8O_7]^{+\cdot}$		264[a] (51) $[M-C_{14}H_{10}O_6]^{+\cdot}$	256 (23) $[274-H_2O]^{+\cdot}$	245 (25) $[274-\dot{C}HO]^{+}$	236 (25) $[264-CO]^{+\cdot}$[b]	
3	274[(a)] (100) $[M]^{+\cdot}$	256 (31) $[M-H_2O]^{+\cdot}$[b]	245 (35) $[M-\dot{C}HO]^{+}$[b]		231 (13) $[M-\dot{C}OCH_3]^{+}$	228 (19) $[256-CO]^{+\cdot}$[b]	203 (12) $[231-CO]^{+}$[b]	200 (15) $[228-CO]^{+\cdot}$[b]	190 (6) $[M-C_4H_4O_2]^{+\cdot}$
4	260 (100) $[M]^{+\cdot}$	232 (54) $[M-CO]^{+\cdot}$[b]	231 (11) $[M-\dot{C}HO]^{+}$		203 (6) $[232-\dot{C}HO]^{+}$[b]	190 (15) $[232-C_2H_2O]^{+\cdot}$[b]			
5	264 (100) $[M]^{+\cdot}$	236 (52) $[M-CO]^{+\cdot}$[b]	218 (15) $[236-H_2O]^{+\cdot}$[b]		205 (19) $[M-CO_2CH_3]^{+}$	177 (36) $[205-CO]^{+}$[b]	149 (51) $[177-CO]^{+}$[b]		
6	520 (100) $[M]^{+\cdot}$	286 (48) $[M-C_{11}H_6O_6]^{+\cdot}$	274 (41) $[M-C_{12}H_6O_6]^{+\cdot}$		273 (33) $[M-\dot{C}_{12}H_7O_6]^{+}$	250 (32) $[M-C_{14}H_6O_6]^{+\cdot}$	236 (31) $[M-C_{15}H_8O_6]^{+\cdot}$		

[a] Elemental composition determined by exact mass measurements.
[b] Fragmentation process demonstrated by a metastable peak.
[c] Mass spectrum obtained at 18 eV.

Table 2. NMR data of purpuromycin and related compounds in $CDCl_3$

Compound	3′–H	6′–H	3–H	4–H	4–OH	5–H	6–H	9′–OH	4′–OH	1′–OH	10–OH	7′–OCH_3	7–CO_2CH_3	acetyl groups
1[a]	2H, 3·46, 3·84 2d J_{gem} = 18	6·16* s	2H, 2·7 m	4·8 m	4·3	7·30 s	7·48 s	11·13	12·11	13·03		3·93	3·96	—
Tetraacetyl I[d]	2H, 3·45, 3·67 2d J_{gem} = 18	6·03 s	2H, 2·75 m	6·2 m	—	7·65 s	7·48 s	—	—	—	—	3·89	3·99	3H, 2·15 6H, 2·35 3H, 2·46
1a[b]		6·15 s			—	6·95 s	7·40 s	11·02	12·25	13·03		3·92	3·95	—
3[c]	1H, 6·63 q J_{all} = 1	6·51* s	3H, 2·50 d J_{all} = 1	—	—	—	—	13·18	13·32				3·96	—
5[c]	—	—	—	10·13 s	—	7·38 s	7·50 s	10·70	11·12				3·96	—

[a] Recorded at 100 MHz after about 200 scans by using a C–1024 time averaging computer.
[b] Data from Ref 6
[c] Recorded at 100 MHz. The numbering system is referred to that adopted for purpuromycin for ease of the reader.
[d] Recorded at 60 MHz.
*The difference in chemical shift suggests that in compound 3 the first ring is aromatic.

Table 3. NMR data of purpuromycin and related compounds in CF_3CO_2D

Compound	3′–H	6′–H	3–H	4–H	5–H	6–H	7′–OCH_3	7–CO_2CH_3
1[a]	2H, 3·58, 3·82 2d J_{gem} = 18	6·48* s	2H, 2·82, 3·06 2dd J_{gem} = 16	5·28 dd J_{AM} = 4, J_{AN} = 1[b]	7·57 s	7·75 s	3·98	4·07
1a[c]	2H, 3·53, 3·80 2d J_{gem} = 18	6·49 s	2H, 2·59 m	2H, 3·20 m	7·21 s	7·70 s	4·02	4·09
2[d]	1H, 6·71 s	6·97* s	2H, 3·6 m	5·8 m	7·42 s	7·74 s	4·05	

[a] Recorded at 100 MHz, single scan.
[b] AMN system treated as AMX system.
[c] Data from Ref 6.
[d] See note[a]—Table 2. The numbering system is referred to that adopted for purpuromycin for ease of the reader.
*The difference in chemical shift indicates[10] that in compound II the first ring is aromatic.

Table 4. IR spectral data of purpuromycin and related compounds

	Naphthazarin moiety					Furan ring			Spiro linkage	Isocourmarin moiety					
Compound	=CH	OH chelated	OH	C=O chelated	C–O–CH_3	=CH	–C=C–	C–O–C	C–O–C–O–C	OH chelated	OH	C=O isocoumarin	C=O ester	C=O aldehyde	C–O–C isocoumarin and ester
1	3070 (ν) 800 (γ)	2750–2500 (ν) 1450, 1335 (δ) 1210, 1110 (ν C–O)		1610(ν) 1310	1235 995 830				950,930 (ν)	coinciding with the bands of the naphthazarin moiety	3510 (ν)	1685 (ν)	1730 (ν)		1275 1220 1150 1095
2	3100 (ν) 800 (γ)	2750–2500 (ν) 1450, 1340 (δ) 1215, 1110 (ν C–O)		1610 (ν) 1310	1245 1000 840	3100 (ν) 710 (γ)	1520 (ν)	970,870 (ν)		coinciding with the bands of the naphthazarin moiety	3570, 3360 (ν) 1035 (ν C–O)	1670 (ν)	1725 (ν)		1280 1215 1160 1100
3	3100 (ν) 810 (γ)	2750–2500 (ν) 1430 (δ) 1210, 1115 (ν C–O)		1610 (ν) 1310	1250 1000 850	3130 (ν) 720 (γ)	1535 (ν)	970,880 (ν)							
4	~3100 (ν) 812 (γ)	2750–2500 (ν) 1450 (δ) 1200, 1110 (γ C–O)	3500 (ν) 1280 (δ) 1230, 1060 (ν C–O)	1620 (ν) 1308		~3100 (ν) 715 (γ)	1530 (ν)	965,870 (ν)							
5										2750–2500 (ν) 1450, 1340 (δ) 1120 (ν C–O)	3500 (ν) 1055 (ν C–O)	1670 (ν)	1725 (ν)	1710 (ν)	1275 1220 1160 1100

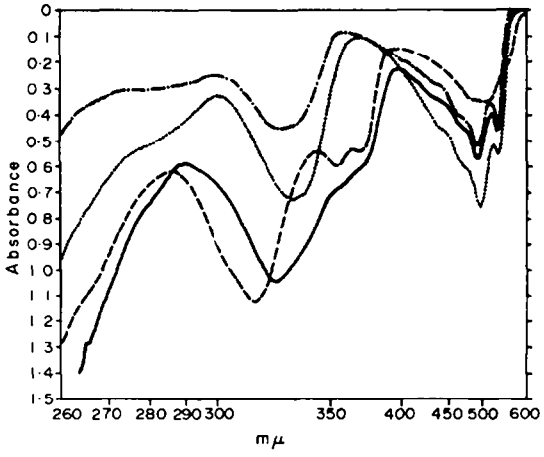


Fig 1. UV and visible absorption spectra in chloroform solution of purpuromycin and related compounds. ---- Compound 1;——Compound 2;-·-·-Compound 3;·····Compound 4.

dation. By heating purpuromycin in pyridine at 100° for 12 h two main products were isolated, 3 and 5, which are related to the two moieties of purpuromycin. The first one is an orange-red crystalline substance which was identified as 4,9 - dihydroxy - 2 - methyl - 7 - methoxy - naphtho[2·3-b]furan - 5,8 - dione (3) in the light of the following evidence. The molecular formula $C_{14}H_{10}O_6$ is obtained from elemental analysis and from the M^+ peak at *m/e* 274 in the mass spectrum (Table 1). The naphthazarin moiety is indicated by the easy reduction of the product (sodium dithionate and zinc powder in dilute hydrochloric acid), by the redox potential value $E_0 = +0{\cdot}055$ V, by the uv and visible absorption maxima (Fig 1), which shifts towards red in alkaline solution and by the ester formation with boracetic anhydride. The two chelated OH groups are at δ 13·18 and 13·32 (Table 2). The presence of the furan ring is indicated by the IR frequencies at 3130, 1535, 970, 880, 720 cm^{-1} (Table 4). The chemical shift of the proton at δ 6·63 and its allylic coupling with the Me group at δ 2·50 (Table 2) indicate the α-substitution at the benzofuran ring. The peak at *m/e* $[M-18]^{+\cdot}$ in the mass spectrum (Table 1) is in accordance for a OMe group *ortho* to the CO in the naphthazarin moiety[8] and suggests the reported tautomeric form with the first ring in quinonic form. Loss of water is observed also from the ions of mass 274 (corresponding to the naphthazarin moiety) present in the spectra of **1** and **2** and therefore the same tautomeric form has been written for all the compounds. Anyhow the existence of the other tautomeric form for compounds **2** and **3** could be deduced from NMR values in different solvents (Tables 2 and 3). The assignment of the OMe group to position 7 stems from the origin of **3** from purpuromycin, for which this position is defined by the NMR signals of the naphthazarin moiety in comparison with those of γ-rubromycin (Table 2).

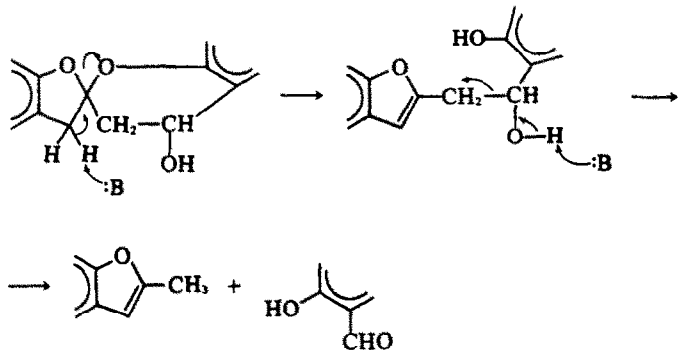
The second degradation product is an orange-yellow crystalline substance for which structure **5** is proposed on the basis of the following evidences. The molecular formula $C_{12}H_8O_7$ is obtained from elemental analysis and from the M^+ peak at *m/e* 264 in the mass spectrum (Table 1). The IR spectrum (Table 4) indicates an aldehydic group (1710) an ester group (1725, 1275, 1160) and another CO function (1670 cm^{-1}). The ^1H NMR spectrum (Table 2) shows the presence of two chelated OH groups (δ 11·12 and 10·70), an aldehydic proton (δ 10·13), tow uncoupled aromatic hydrogens (δ 7·50 and 7·38) and a OMe group (δ 3·96). The functionalities described leave two carbons and one oxygen with two unsaturations to be accounted for and therefore a 6-membered ring lactone fused to the aromatic has to be considered. The two OH groups are in *ortho* position as indicated by the formation of a diphenylmethane - dioxy derivative with dichloro diphenyl methane[9] and adjacent to a CO as indicated by their chemical shift. The aldehydic function has to be *ortho* to an OH group to explain the isomerisation reaction described later. The position of the carbomethoxy group has been assigned considering the origin of **3** from purpuromycin. The IR 1670 cm^{-1} absorption and the ions in the mass spectrum (Table 1) corresponding to losses of three CO groups (confirmed by metastable peaks) and to the loss of one carbomethoxy group are in accordance with structure **5**.

Compound **3** was obtained from purpuromycin also by alkali fusion but in this case the major product was the demethoxylated compound **4**. The mass spectrum (M^+ at *m/e* 260) does not show the loss of water from the molecular ion, as expected because of the absence of the OMe group. The IR spectrum presents the absorptions at 1530, 965, 870 and 715 cm^{-1} typical of the furan ring and the absorption of a free OH group (3500 cm^{-1}). ^1H NMR and UV spectra are in accordance for structure **4**.

The isomerisation described for γ-rubromycin with formation of **2a**[6] was observed also for purpuromycin. Thus, by treating purpuromycin with pyridine at 70° for 2·5 h, a product of isomerisation was isolated to which structure **2** was assigned on the basis of its chemico-physical data described as follows in comparison with purpuromycin. The UV spectrum (Fig 1) shows a bathochromic and hyperchromic shift of the maxima, thus revealing a further conjugation of the naphthoquinone chromophore; furthermore, the visible maxima are identical to those of compounds **3** and **4** both containing the naphthofuran system. The IR spectrum presents absorptions at 3360 (additional OH group) and at 1520, 970, 870, 710 cm^{-1}

(furan ring). The ^1H NMR spectrum (Table 3) reveals the appearance of an additional aromatic proton and the disappearance of the methylene group at δ 3·58 and 3·82. The mass spectrum shows the same general fragmentation pathway (Table 1) observed for purpuromycin and is in accordance for structure **2**.

Iso-purpuromycin heated in pyridine for 12 h yielded compound **3**, showing that **3** derives from purpuromycin through iso-purpuromycin. The two base catalysed processes can be formulated as follows:



The OH group present in purpuromycin favors the cleavage, in basic conditions, at the C_3–C_4 bond via a retro aldol reaction, thus explaining the formation of **2** and **5**. The origin of the peak at *m/e* 264 in the mass spectrum of purpuromycin (Table 1) was also understood as a retro aldol type cleavage and corresponds to **3**.[7] γ-Rubromycin, in which the C_3–C_4 bond is stable, undergoes in alkali conditions a benzylic cleavage at C_2–C_3 bond with formation of a substituted naphthazarine.[10]

The final confirmation of structure **1** was given by obtaining micro amounts of γ-rubromycin starting from purpuromycin in the following way. Purpuromycin was dehydrated by thermal decomposition at 240° to yield 3,4-anhydropurpuromycin **6** and then catalytically reduced to give γ-rubromycin. The product obtained resulted identical (R_f chromatographic value and mass spectrum) with an authentic sample.

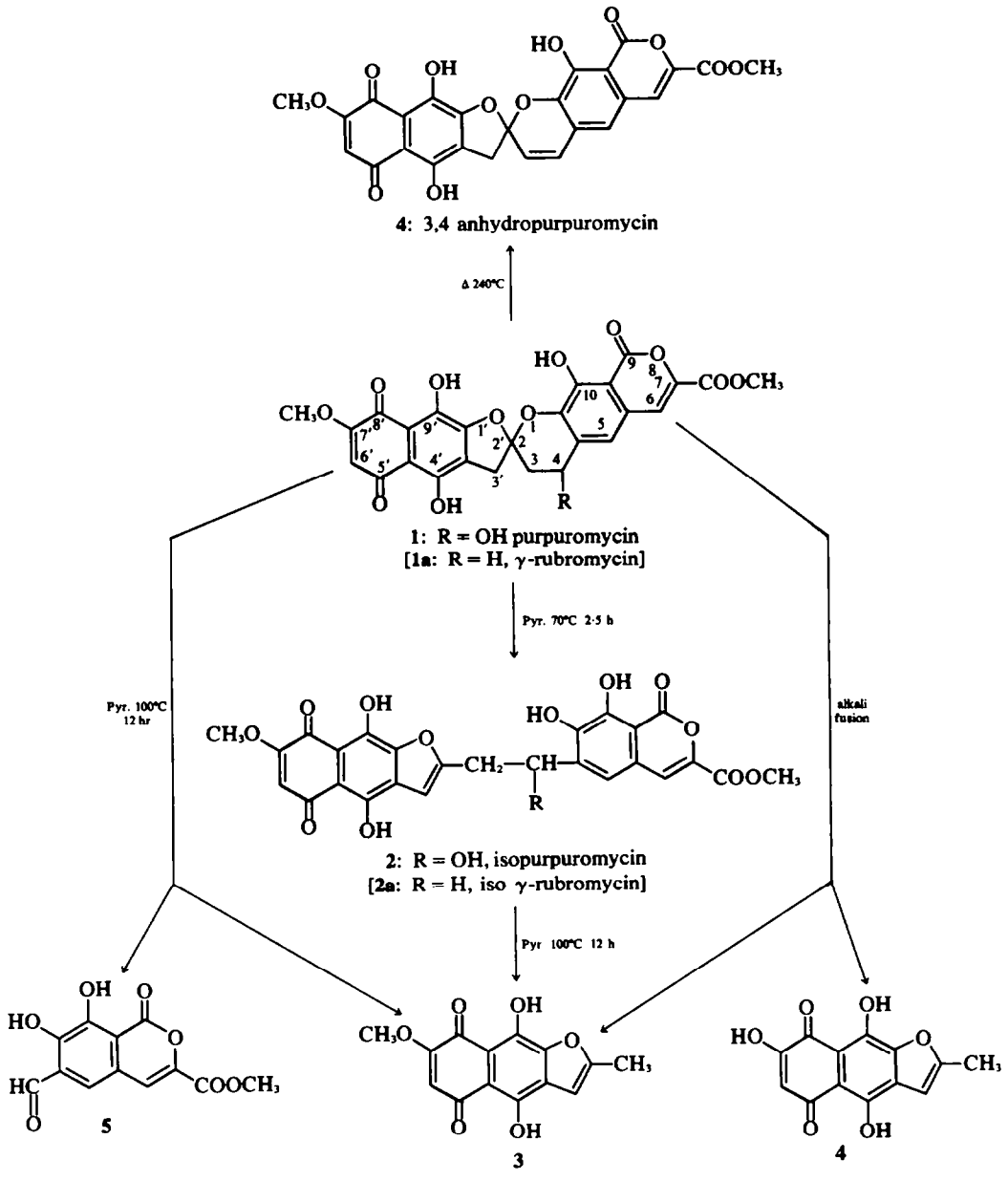
EXPERIMENTAL

All m.ps were determined in open capillaries and are uncorrected. UV spectra were recorded on a P.E. spectracord 4000–A instrument. IR spectra were recorded on a P.E. Mod. 425 instrument in Nujol mull. ^1H NMR spectra were recorded at 60 MHz on a Varian A–60D spectrometer and at 100 MHz on a Varian XL—100—15 spectrometer. Chemical shifts are expressed in ppm downfield from TMS as internal reference. Mass spectra were recorded on Hitachi P. E. RMU-6L and P.E. 270 spectrometers at 70 eV, using the direct inlet system heated at 160–300°. Exact mass measurements were performed with a double focusing Hitachi-P.E. RMU–6D instrument by the peak matching technique. Thin layer chromatographic analyses were performed over Silica-gel plates HF_{254}-E. Merk buffered with 0·5 N KH_2PO_4 using ascending technique in $CHCl_3$:MeOH mixtures.

Purpuromycin. The isolation of purpuromycin from *Actinoplanes ianthinogenes* and its purification have been described in a previous paper.[1] Purpuromycin was crystallised from $CHCl_3$ containing 1–5% MeOH. It decomposes at 212° and doesn't melt up to 320°. R_f = 0·32 (eluent $CHCl_3$/MeOH 98/2; 0·55 (eluent $CHCl_3$/MeOH 95/5). (Found: C 57·90; H 3·38, $C_{26}H_{18}O_{13}$ requires: C 58·0; H 3·35%) Polarographic $E_{1/2}$ = −0·647 volt *vs* SCE pH 9·8. λ_{max} ($CHCl_3$): 300 (sh), 313 (log ε = 4·37), 352 (4·09), 368 (4·06), 480 (sh), 505 (3·86), 545 (sh) nm: λ_{max} (HCl 0·1N): 312 (log ε = 4·02), 360 (sh), 495 (3·67), nm; λ_{max} (NaOH 0·1N): 234 (log ε = 4·55), 260 (sh), 324 (4·08), 382 (3·98), 555 (3·98), 585 (4·02), nm.

Tetra-acetyl purpuromycin. A suspension of purpuromycin (300 mg) in anhyd pyridine (10 ml), to which Ac_2O (16 ml) was added, was heated for 1 h at 75°; the soln obtained was kept at room temp for 48 h, then poured into an ice-water mixture and the ppt was collected. The product obtained was separated by column chromatography (support: silicagel, eluents: $CHCl_3$ and $CHCl_3$—MeOH mixtures). Tetra-acetyl purpuromycin was eluted with the $CHCl_3$/MeOH, 98/2 mixture and crystallised from acetone (mg 120). R_f = 0·44 (eluent $CHCl_3$/MeOH, 98/2), m.p. = 250–254°. (Found C, 57·12; H, 3·67; $C_{34}H_{26}O_{17}$ requires: C, 57·8, H, 3·69%); λ_{max} ($CHCl_3$): 262 (sh), 283 (sh), 292 (log ε = 4·56), 307 (sh), 384 (4·09), 360 (sh), nm; λ_{max} (HCl 0·1 N): 241 (log ε = 3·80), 260 (3·47), 293 (3·67), 300 (sh), 342 (3·26), nm; λ_{max} (NaOH 0·1 N: 235 (sh), 320 (log ε = 3·29), 329 (sh), 545 (3·10), 580 (3·10), nm.

Compounds 3 and 5 from purpuromycin by pyridine treatment. A suspension of purpuromycin (500 mg) in anhyd pyridine (50 ml) was heated for 12 h at 100°. The soln obtained was poured into an ice-water mixture and after acidification the ppt was collected. The filtrate was extracted three times with EtOAc and the solvent was evaporated to dryness, obtaining a residue. The ppt was suspended in 300 ml of acetone and stirred for 1 h at room temp. The insoluble fraction (120 mg) was identified as isopurpuromycin, and the acetonic soln was evaporated to dryness. The residue obtained and the residue previously obtained from EtOAc were collected and purified by column chromatography (support silicagel buffered with KH_2PO_4 0·5 N). Elution with $CHCl_3$ and crystallization from chloroform containing 5% MeOH yielded compound



SCHEME

3 (60 mg). $R_f = 0{\cdot}71$ (eluent $CHCl_3$/MeOH, 98/2) m.p. = 300°. (Found: C, 60·97; H, 3·72; $C_{14}H_{10}O_6$ requires: C, 60·65; H, 3·72%). Polarographic $E_{1/2} = 0{\cdot}765$ *vs* SCE pH 9·8. λ_{max} (HCl 0·1 N): 292 (log ϵ = 3·94), 330 (3·93), 494 (3·73), 548 (3·57), nm; λ_{max} (buffer pH 7·0): 338 (log ϵ = 4·01), 516 (3·92), 553 (3·95), nm; λ_{max} (NaOH 0·1 N): 330 (log ϵ = 4·02), 526 (4·08), 556 (4·14), nm.

Compound **5** (10 mg) was obtained by elution with $CHCl_3$/MeOH 99/1 mixture and was crystallized from chloroform with 1–5% of MeOH, $R_f = 0{\cdot}53$ (eluent: $CHCl_3/CH_3OH$ 95/5) m.p. = 216–218°. (Found: c, 54·03; H, 3·22; $C_{12}H_8O_7$ requires: C, 54·54, H, 3·05); λ_{max} ($CHCl_3$): 287 (log ϵ = 3·90), 301 (3·86), 315 (3·87), 392 (sh), 414 (3·41), 438 (sh), nm.

Compounds **3** *and* **4** *from purpuromycin by alkali fusion.* Purpuromycin (450 mg) was mixed with NaOH (2·5 g) and KOH (2·5 g) pellets. This mixture was poured into a sealed Ni tube and heated at 150° for 45 min. The mixture was then dissolved into water (70 ml) and the violet soln obtained was acidified with HCl 5N to pH 2. The suspension was saturated with NaCl and the red ppt was collected. The filtrate was extracted with ethyl ether 5 times and the combined extracts evaporated to dryness under vacuum. The residue (50 mg) and the red ppt (310 mg) were suspended into ether (50 ml) and stirred for 4 h at room temp. After filtration of the insoluble fraction, the filtrate was evaporated to dryness and the residue obtained (60 mg) separated by column chromatography

(support silicagel buffered with KH_2PO_4 0·5 N; eluent $CHCl_3$, fractions volume: 10 ml). Compound 3 (mg 10) was obtained in crystalline form from the first 10 fractions R_f = 0·71 (eluent $CHCl_3$/MeOH 98/2). (Found: C, 60·92, H, 3·70, $C_{14}H_{10}O_6$ requires: C, 60·65, H, 3·72%).

Compound 4 was obtained from the fractions 15 to 25 and crystallized from $CHCl_3$ with 1–5% MeOH (mg 27). R_f = 0·24 (eluent: $CHCl_3$/MeOH, 98/2), m.p. 250–255°, λ_{max} ($CHCl_3$): 331 (log ϵ = 3·49), 440 (sh), 470 (sh), 495 (3·96), 530 (3·82), nm, λ_{max} (HCl 0·1 N): 241, (log ϵ = 3·69), 332 (3·41), 435 (3·37), nm; λ_{max} (NaOH 0·1 N): 257 (log ϵ = 3·68), 312 (3·51), 348 (3·56), 515 (3·77), 555 (3·76), nm; ^{1}H NMR spectrum (60 MHz C_5D_5N) 6·82 s, 1 H), 6·72 (q, J = 1 Hz, 1 H), 2·28 (d, J = 1 Hz, 3H).

Isopurpuromycin from purpuromycin. Purpuromycin (50 mg) in anhyd pyridine (6 ml) was warmed until a clear soln was obtained and kept for 2·5 h at 70°. The soln was poured into ice-water mixture, the ppt formed filtered and washed with $CHCl_3$, $CHCl_3$-MeOH 95–5 mixture and acetone. Isopurpuromycin (33 mg) was obtained as residue almost insoluble in the majority of organic solvents. m.p. = >320·. (Found: C, 56·51; H, 3·41; $C_{26}H_{18}O_{13}$ requires: C, 58·0; H, 3·35%). Polarographic $E_{1/2}$ = 0·655 volt *vs* SCE pH 9·8, λ_{max} ($CHCl_3$): 530 nm (log ϵ = 3·96); 490 (4·06); 465 sh 440 sh; 370 sh; 322 (4·32); λ_{max} (HCl 0·1 N): 535 (3·78); 500 (3·88); 324 (4·11); λ_{max} (NaOH 0·1 N): 575 (4·08); 535 (4·09); 415 (4·14); 332 (4·10).

Compound 3 from isopurpuromycin. A suspension of isopurpuromycin (30 mg) in anhyd pyridine (2 ml) was heated at 100° for 12 h. The soln was poured into ice-water mixture and the ppt obtained after acidification was extracted with $CHCl_3$; the $CHCl_3$ extracts were concentrated to dryness and the product obtained was purified by column chromatography as described previously.

γ-Rubromycin from purpuromycin. 50 mg of purpuromycin were heated at 240° for 4 h under N_2 in a thermoanalyzer Du Pont 900. 10 mg of the dehydrated product (6) were obtained after TLC purification R_f = 0·66 (eluent $CHCl_3$/MeOH, OH, 95/5). (Found: C, 60·13 H, 3·10 $C_{26}H_{16}O_{12}$ requires: C, 60·02; H, 3·10). This product was suspended in $CHCl_3$/MeOH 9/1 and hydrogenated on 10% Pd/C at room pressure and temp for 1 h. After filtration and evaporation, the residue obtained presented a mixture of products on TLC analysis and γ-rubromycin was obtained in micro amount after TLC separation. R_f = 0·9 (eluent $CHCl_3$/MeOH 95/5). M^+ at *m/e* 522.

Acknowledgements—We thank Dr. P. Ferrari for IR and UV spectra, Mr. M. Nebuloni for thermal analysis. We are indebted to Dr. G. G. Gallo and Dr. G. C. Lancini for helpful discussions and criticism.

REFERENCES

[1]C. Coronelli, H. Pagani, M. R. Bardone and G. C. Lancini, *J. Antibiotics.* Int. Ed. **27**, 161–168 (1974)
[2]W. M. Clark, *Oxidation-reduction potentials of organic systems.* Williams and Wilkins, Baltimore (1960)
[3]O. Dimroth and T. Faust, *Ber. Dtsch. Chem. Ges.* **54**, 3020 (1921)
[4]H. Raistrick, R. Robinson and A. R. Todd, *Biochem. J.* **28**, 559 (1934)
[5]H. Brockmann and K. H. Reuneberg, *Naturwiss* **40**, 59 (1953)
[6]H. Brockmann, W. Lenk, G. Schwantje and A. Zeeck, *Chem. Ber.* **102**, No. 1, 126 (1969)
[7]L. F. Zerilli, M. Landi, G. G. Gallo and M. R. Bardone, (Edited by E. Frigerio and N. Castagnoli) p. 235, Raven Press, New York (1974)
[8]D. Becher, C. Djerassi, R. E. Moore, H. Singh and P. I. Scheuer, *J. Org. Chem.* **31**, 3650 (1966)
[9]L. Jurd, *J. Am. Chem. Soc.* **81**, 4606 (1959)
[10]H. Brockmann and A. Zeeck, *Chem. Ber.* **103**, 1709 (1970)