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SYNTHESIS AND STRUCTURE DETERMINATION OF SOME DERIVATIVES OF THE ANTIBIOTIC THERMORUBIN

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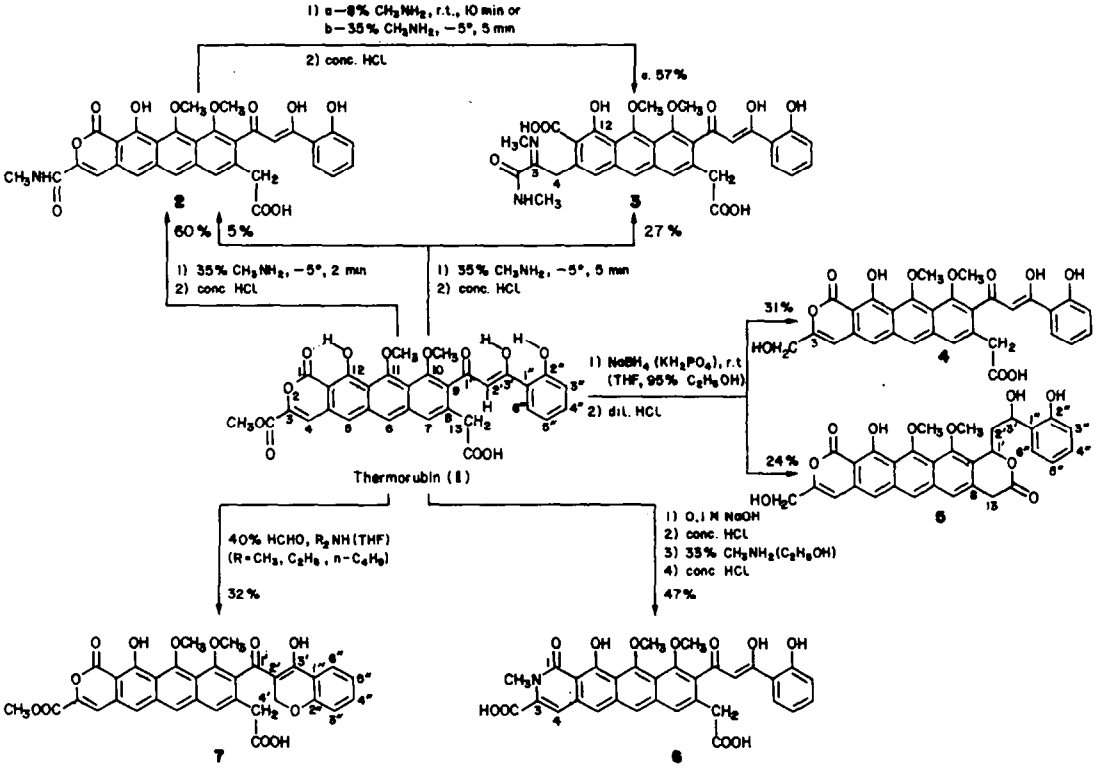
Abstract—Reactions of thermorubin (**1**), an antibiotic produced by *Thermoactinomyces antibioticus*, with conventional reagents such as CH_3NH_2, $NaBH_4$, $HCHO/R_2NH$ were studied. Several derivatives were obtained whose structures were determined on the basis of chemical and spectroscopic data. Some compounds showed strong modifications of the original structure.

Thermorubin (**1**), an antibiotic produced by *Thermoactinomyces antibioticus*,[1] is active *in vitro* against bacteria by specific inhibition of protein synthesis.[2] The structure originally indicated as a xanthone-methylene-anthracene[3] was shown to be incorrect by Johnson *et al.*,[4] who definitely assigned a phenyl-propenyl-oxanaphthacenone structure (Scheme 1). The molecule is characterized by the presence of an unsaturated lactone ring condensed with an anthracenic moiety (carrying an acetic acid residue) which is linked through an enolizable 1,3-diketo system to a phenol.

The formula $C_{32}H_{24}O_{12}$ corresponds to the molecular weight 600.54 daltons. In a previous paper[4] it



Scheme 1.

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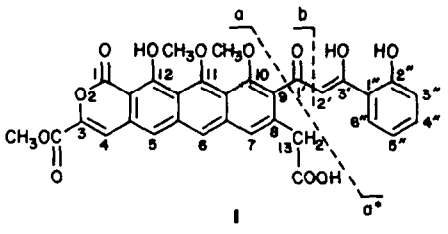
was observed that neither **1** nor its trimethyl derivative give the molecular ions in the mass spectra under electron impact but only the $M+14]^+$ ions deriving from an intermolecular transfer of a methyl group. The mass spectra taken by LC/MS in both positive and negative chemical ionization gave the $M+H]^+$ and M^- molecular ions. The details of this procedure and

Table 1. LC/MS data of thermorubin (1) and its derivatives[a]



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Compound	Significant ions (m/z) M+H+14]+	M+H]+	b]+	a+2]+	a*]+
1	615	601	465	439	163
2	614	600	464	438	163
3	645	631	495	469	163
4	587	573	437	411	163
5	573	559	421	—	—
6	614	600	464	438	163
7	627	613	465	439	175

[a] See Experimental.

the results will be presented in a subsequent paper,[5] while the data relevant for the determination of the structures discussed here are shown in Table 1. Thus, 1 exhibits the M+H]+ molecular ion at m/z 601 together with the M+H+14]+ peak at m/z 615 and a series of diagnostic fragments. The M⁻ ion at m/z 600 is clearly detected in negative ionization.[5]

Following the determination of the structure, a program for the chemical modification of thermorubin was established in an attempt to prepare derivatives with enhanced antibacterial activity. It was found that treating it with conventional reagents strong modifications of the original structure occurred (Scheme 1).

(1) By allowing 1 to react at −5° with an excess of aqueous CH_3NH_2 for 5 min followed by acidification a mixture of the amide 2 (5%) and 3 (27%) containing two N atoms was obtained. If the reaction is stopped after 2 min the presence of 2 is predominant. However, 2 is transformed into 3 at −5° by 35% aqueous CH_3NH_2 for 5 min or 8% aqueous CH_3NH_2 for 10 min at room temperature.

(2) The reduction of 1 with a large excess of $NaBH_4$ in the presence of KH_2PO_4 at room temperature in THF gives the corresponding alcohol 4 together with 5 which, besides the alcoholic function, carries a new lactone ring originating from the reduction of the 1,3-diketo system and cyclization with the acetic moiety. Two diastereoisomers are expected to originate from the reduction of the 1,3-diketo system. Given the homogeneity of the physicochemical characteristics, 5 is assumed as one diastereoisomer, *threo* or *erythro*: which of the two was not investigated.

(3) Treatment of 1 with 0.1 N NaOH followed by acidification yields the corresponding diacid which on reacting with 33% ethanolic CH_3NH_2 for 10 mn at room temperature gives after acidification 6 containing an unsaturated lactam ring instead of the original lactone.

(4) Compound 1 reacts with formaldehyde and a series of secondary amines (dimethylamine, diethylamine and di-n-butylamine) under the conditions of the Mannich reaction affording 7 in which an α-pyran ring is generated by condensation between the phenolic hydroxyl and the aminomethyl group on the 1,3-diketo system, independently from the amine used.

The structures proposed hereafter are based upon elemental analysis, MS, ^{1}H-NMR, IR and UV-VIS data, and acid–base titrations, which are reported in Tables 1–5 in comparison with the data for thermorubin (1).

Compound 2. All the data are in accordance with the transformation of the carboxymethyl in position 3 into the methylamide. In particular, the ^{1}H-NMR spectrum shows all the signals present in the spectrum of 1 (Fig. 1), except for the absence of the CH_3O—CO singlet at δ 3.95 ppm, and the presence of the CH_3—NH—CO signals at δ 2.80 and 8.60 ppm. The small singlet at δ 4.81 ppm in the spectrum of thermorubin (1) is attributed to the CH_2-2′ of the tautomeric 1,3-diketo form (20%). This signal is absent in the spectrum of 2.

The UV spectrum of 2 practically corresponds to that of 1 and this is indicative that there is no variation in the basic chromophoric system.

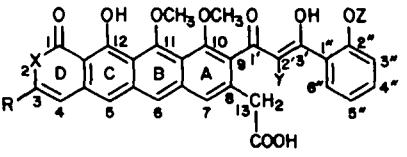
The pK_a values determined in DMF/H_2O, 10:1 (v/v) of 2 are the same as those of 1 (Table 5), confirming that these functions have remained unchanged. It is worthwhile mentioning that the higher values obtained in the present case in comparison with the previous ones (4.7, 7.1 and 9.1)[6] are due to the use of the solvent system DMF/H_2O, 10:1 instead of C_2H_5OH/H_2O, 1:9.

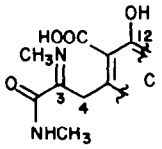
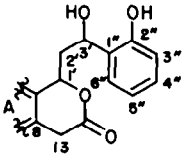
Table 2. Physicochemical characteristics of thermorubin (1) and its derivatives

Compound	Yield %	TLC, R_f[a] $CHCl_3$–MeOH 9:1	M.p.[b] °(dec)	Formula	MW	Elemental analysis[c]
1	—	0.56 orange	>270	$C_{32}H_{24}O_{12}$	600.54	C,H
2	60	0.52 orange	>270	$C_{32}H_{25}NO_{11}$	599.60	C,H,N
3	27 from 1 57 from 2	0.40 yellow	>270	$C_{33}H_{30}N_2O_{11}$	630.62	C,H,N
4	31	0.47 orange	165–168	$C_{31}H_{24}O_{11}$	572.53	C,H
5	24	0.49 red	168–170	$C_{31}H_{26}O_{10}$	558.55	C,H
6	47	0.0 orange	>270	$C_{32}H_{25}NO_{11}$	599.56	C,H,N
7	32	0.65 yellow	>270	$C_{33}H_{24}O_{12}\cdot\frac{1}{2}CH_2Cl_2$	655.01	C,H,Cl

[a] See Experimental.
[b] M.ps (uncorrected) were determined in glass capillary tubes.
[c] The analytical results were within ±0.4% of the theoretical values.

Table 3. ^{1}H-NMR data for thermorubin (1) and its derivatives at 270 MHz in DMSO-d_6 (δ in ppm, J in Hz)[a,b]



	1	2	4	6	7	3	5
							
Protons	R = $COOCH_3$ X = O Y = Z = H	R = $CONHCH_3$ X = O Y = Z = H	R = CH_2OH X = O Y = Z = H	R = COOH X = NCH_3 Y = Z = H	R = $COOCH_3$ X = O (YZ) = CH_2	Y = Z = H	R = CH_2OH X = O
H-4	7.77, s	7.75, s	6.70, s	7.85, s	7.90, s	3.25, 3.48, 2d, CH_2-4 (16)	6.60, s
H-5	7.63, s	7.45, s	7.47, s	7.48, s	7.78, s	7.20, s	7.32, s
H-6	8.28, s	8.30, s	8.20, s	8.37, s	8.42, s	8.10, s	8.05, s
H-7	7.82, s	7.83, s	7.77, s	7.87, s	7.95, s	7.73, s	7.57, s
CH_2-13	3.80, s	3.82, s	3.80, s	3.83, s	3.83, s	3.77, s	3.80, 4.08, 2d (19)
OCH_3-10	3.87, s	3.90, s	3.88, s	3.93, s	3.93, s	3.87, s	3.98, s
OCH_3-11	3.90, s	3.95, s	3.95, s	3.98, s	3.93, s	3.90, s	4.00, s
OH-12	13.25, s	13.30, s	13.43, s	12.85, s	3.32, s	7.26, s	13.63, s
Y-2′	7.02, s	7.00, s	7.00, s	7.02, s	—	6.97, s	2.08, 2.45, 2 ddd, CH_2-2′, J_{gem} 12, J_{vic} 2.5, 11 J_{vic} 0.5, 11
OH-3′	16.01, s	16.00, s	16.10, s	16.28, br	15.80, br	16.08, br	5.17, d (4.5)
OZ-2″	11.03, s	11.03, s	11.03, s	11.10, s	—	11.03, br	8.55, s
H-3″	6.98, m	6.97, m	6.98, m	7.03, m	7.02, d (8.5)	6.95, m	6.65, d (8.5)
H-5″	6.98, m	6.97, m	6.98, m	7.03, m	7.60, dd (8.5)	6.95, m	6.98, dd (8.5)
H-4″	7.45, dd (8.5)	7.43, dd (8.5)	7.45, dd (8.5)	7.48, dd (8.5)	7.20, dd (8.5)	7.43, dd (8.5)	6.75, dd (8.5)
H-6″	7.92, d (8.5)	7.95, d (8.5)	7.92, d (8.5)	7.97, d (8.5)	7.90, s	7.88, d (8.5)	7.33, d
COOH	12.5 br	12.5, br	12.5, br	12.95, br	12.55, br	12.43 br	—
R	3.95, s, 3H	2.80, d, 3H (5.5)	4.30, s, $C\underline{H}_2OH$	15.72, br, 1H	4.0, s, 3H	—	4.32, d, $C\underline{H}_2OH$ (6)
	—	8.60, q, 1H (5.5)	5.67, br, $CH_2O\underline{H}$	—	—	—	4.77, t, $CH_2O\underline{H}$ (6)
X	—	—	—	3.63, s, 3H	—	—	—
(YZ)	—	—	—	—	4.72, 4.88, 2bd	—	—
	—	—	—	—	—	2.65, d, CH_3(NH)	5.32, ddd, CH-3′
	—	—	—	—	—	2.92, s, CH_3N	6.37, dd, CH-1′
	—	—	—	—	—	8.27, q, NHCO	—
	—	—	—	—	—	15.35, s, COOH	—

[a] Compound 5 was dissolved in THF-d_8.

[b] b, broad; br, broad singlet; s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; t, triplet; m, multiplet; q, quartet.

Compound 3. The elemental analysis accounts for the presence of two N atoms, confirmed by the molecular ion $M+H]^+$ at *m/z* 631 in the MS spectrum. The presence of a fragment at *m/z* 163, corresponding to the moiety deriving from cleavage of the bond C-9—C-1′, in 1–3 demonstrates that the 1,3-diketo system carrying the phenolic ring is not modified.

The ^{1}H-NMR spectrum of 3 shows the signals of the amide at δ 2.65 and 8.27 ppm, respectively, and a singlet at δ 2.92 ppm attributable to a C=N—CH_3 group. In addition, two doublets at δ 3.25 and 3.48 ppm assigned to a CH_2 possessing two non-equivalent hydrogens and the shift from δ 13.30 ppm in 2 to δ 7.26 ppm in 3 of the signal corresponding to the OH-12 suggest a modification of the lactone portion of the molecule. This latter shift is probably due to the fact that in 2 there is a strong intramolecular H-bond between OH-12 and C-1=O allowed by the rigid geometry of the molecule[4] while in 3 the intramolecular bond between the carboxyl group and the OH-12 is weaker and probably broken by the solvent.

In the IR spectrum the disappearance of the ν C=O band of the lactone at 1660 cm^{-1} in 2 confirms the modification in this part of the molecule while the absorption at 1655 cm^{-1} indicates an *o*-hydroxy substituted benzoic acid. The band at 1680 cm^{-1} (amide I) in 2 is shifted to 1630 cm^{-1} in 3. The acid–base titration also is in accordance.

It might be supposed that after the amidation of the ester the methylamine attacks position 3 opening the α-pyrone ring; subsequent tautomerization to the more stable imine form affords 3 (Scheme 2). The reaction

Table 4. Characteristic IR frequencies (cm^{-1}) of thermorubin (1) and its derivatives[a]

Compound	νNH	νOH	νC=O ester	νC=O carboxyl	νC=O lactone	Amide I	νC2'=C3', νC1'=O	νC=C	Amide II	νC—O	γC—H arom
1	—	2720, 2610	1733	1703	1662	—	1630, 1610	1580, 1550, 1490	—	1225, 1125	760
2	3340	3200–2500	—	1725	1660	1680	1620, 1610	1580, 1490	1550	1080	760
3	3360	3300–2500	—	1725,	—	1630	1615	1580,	1555	1100	755
			—	1655 (C1=O)				1605	1490		
4	—	3700–3300, 3300–2500		1695	1680	—	1615	1585, 1550, 1500	—	1245, 1160, 1100, 1040	765
5	—	3600–2500	—	—	1745, 1680	—	—	1555, 1490	—	1230–1090	750
6	—	3400–2500	—	1735, 1720	—	1620 (C1=O)	1620, 1600	1585, 1555, 1500	—	1200, 1120, 1035	760
7	—	3400–3200, 2800–2500	1740	1730	1670	—	1615	1555	—	1230, 1170, 1125	760, 730

[a] See Experimental.

between 2 and methylamine to give 3 deserves a further comment. It is known that α-pyrones react easily with nucleophiles: the attack is reported to occur at positions 2, 4 and 6, position 2 being preferred by strong nucleophiles and position 6 by weak ones.[7] For example, α-pyrones react with sodium cyanide in DMF to give the corresponding muconic acid mononitriles through the ring opening.[8] In the present case, position 3 (corresponding to position 6 in α-pyrones) is more activated than position 1 probably due to the presence of the amidic carbonyl, and this fact may explain the observed reactivity.

Compound 4. It shows all the relevant NMR data of 1 except for the absence of the CH_3O—CO singlet at

Table 5. Acid–base titration and UV data for thermorubin (1) and its derivatives

Compound	Acid–base titration[a] Ionizable functions	$pH_{\frac{1}{2}}$	Attribution	UV[b] λ_{max}, nm	ε_{max}
1	1	7.8	C-13 carboxyl	297	55,102
	2 and 3[b]	10.4	phenol and enolic system	330	51,110
				430	51,173
2	1	7.8	C-13 carboxyl	298	61,497
	2 and 3[c]	10.4	phenol and enolic system	327	57,880
				425	17,183
3	1	7.8	C-13 carboxyl	277	51,204
	2 and 3[c,d]	9.7	phenol and enolic system	297	49,856
				355	15,720
				385	13,923
4	1	7.6	C-13 carboxyl	297	51,926
	2[c]	10.5, 12.8	phenol and enolic system	355	24,172
				400	15,219
5	1	11.2	phenol	300	90,469
	2 (and 3)[c]	13.5	phenol	375	7867
				395	14,750
6	1	6.0	C-3 carboxyl	305	58,861
	2	7.8	C-13 carboxyl	330 (sh)	44,734
	3 and 4	10.0	phenol and enolic system	418	20,259
7	1	7.6	C-13 carboxyl	296	59,956
	2, 3 and 4	9.85	2 phenols and lactone ring	328	56,958
				425	17,987

[a] Acid–base titration in DMF–H_2O, 10:1 (in volume) with 0.1 N KOH.
[b] The compounds were dissolved in 95° EtOH containing 1% of DMF.
[c] This titration may be disturbed by the opening of the lactone ring.
[d] An excess of HCl was added before the titration with 0.1 N KOH.

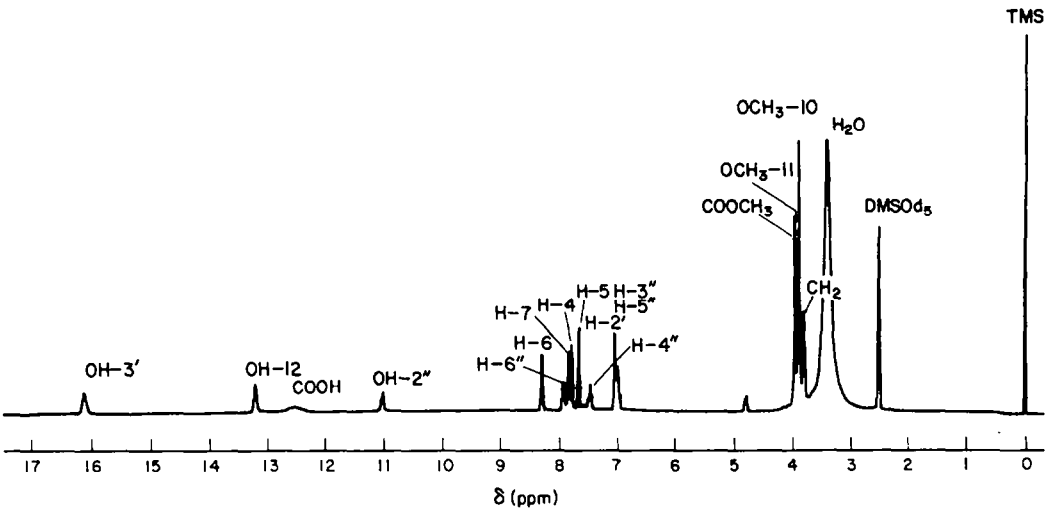


Fig. 1. ^{1}H-NMR spectrum (270 MHz, DMSO-d_6) of thermorubin (**1**).

δ 3.95 ppm and the presence of signals at δ 4.30 and 5.67 ppm due to the CH_2OH group in position 3. No coupling constant (J) is observed between the protons of this group probably because of the presence of the acetic acid moiety, which promotes chemical exchange.

Compound **5**. The formula $C_{31}H_{26}O_{10}$ was confirmed by the $M+H]^+$ peak at *m/z* 559 in the mass spectrum. In this case, the absence of the fragment at *m/z* 163 suggests that the right part of the molecule is modified.

The ^{1}H-NMR spectrum shows all the signals of the anthracenic skeleton (considering the variations of the δ values due to the different solvent) and the signals of the CH_2OH in position 3 found for **4**, although in this case a coupling constant J = 6 Hz is observed. The signals corresponding to the CH_2-13, H-2' and OH-3 are absent. On the other hand, two doublets at δ 3.80 and 4.08 ppm are attributed to the two non-equivalent protons of CH_2-13 while signals at δ 2.08 and 2.45 ppm, δ 5.17 and 5.32 ppm, and δ 6.37 ppm form a network of coupled spins suggesting the presence of a —O—CH—CH_2—CH(OH)— unit deriving from the reduction of the 1,3-diketo system.

The absence of the strong absorption band at 1615 cm^{-1} in the IR spectrum of **5** present in the spectrum of **4** again confirms the disappearance of the 1,3-diketone system while the 1745 cm^{-1} band is attributable to an additional lactone.

Combining the above information with the substantial difference of the mass spectrum which does not show the fragmentation patterns of **1** and **4** but a fragment at *m/z* 421 due to the cleavage of the C-1'—C-2' bond the structure shown in Scheme 1 can be assigned to **5**. The acid–base titration is in agreement with the disappearance of the carboxyl group and of the enolic system, and with the presence of two phenolic groups.

Compound **6**. The molecular formula $C_{32}H_{25}NO_{11}$ by elemental analysis shows the presence of a possible NH group instead of an O atom, confirmed by the molecular ion $M+H]^+$ at *m/z* 600 in the mass spectrum. The fragmentation pattern practically corresponds to that of **1**.

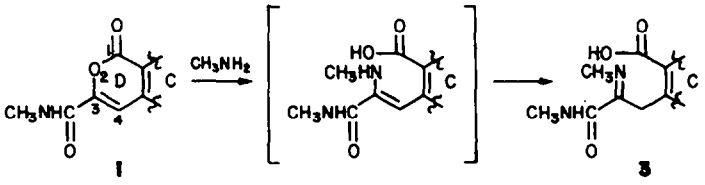
In the ^{1}H-NMR spectrum the only differences between **6** and **1** are the presence in **6** of a signal at δ 15.72 ppm, due to the free COOH in position 3, instead of the $COOCH_3$ signal at δ 3.95 ppm, and a singlet at δ 3.63 ppm attributed to an N—CH_3 group.

The disappearance of the band at 1662 cm^{-1} in the IR spectrum of **1** suggests a modification of the lactone ring.

Therefore, the unsaturated lactam ring is assigned to **6**. The acid–base titration is clearly indicative of the introduction of an additional conjugated carboxyl group.

The transformation of a lactone into a lactam ring has been described for α-pyrones[7] and isocoumarins,[9,10] but under experimental conditions stronger than those applied in the present case.

Compound **7**. This was isolated as an orange crystalline solvate with 0.5 mol of CH_2Cl_2. The elemental analysis gives the formula $C_{33}H_{24}O_{12}$,



Scheme 2.

confirmed by the M + H]$^+$ peak at *m/z* 613 in the mass spectrum. A series of fragments deriving from M + H]$^+$ are found that correspond to those given by **1**. In particular, the fragment designated b]$^+$ in Table 1 being present in both spectra suggests that the anthracenic moiety is unchanged, while the fragment at *m/z* 175 is indicative that the fragment corresponding to the original aryl-1,3-diketone contains an additional C atom.

This is confirmed by the ^{1}H-NMR spectrum of **7** which differs from that of the parent compound by the lack of the H-2′ singlet at δ 7.02 ppm and of the singlet at δ 11.03 ppm attributed to the OH-2″. Besides, a two doublets system at δ 4.72 and 4.88 ppm is attributed to a methylenic group with two non-equivalent protons.

In the IR spectrum all the main bands of the parent compound are present, but a number of shifts suggest that some structure modification took place.

These considerations are consistent with the structure proposed that might result from the elimination of the amine residues from the intermediate Mannich derivatives on C-2′ by attack of the phenolic OH as shown in Scheme 3.

The UV–VIS data, which are shown in Table 5, are of some diagnostic value for structure assignment on the basis of the following discussion. Three bands are reported for each compound, corresponding in the order of increasing wavelength, the first to the *short wavelength* band (λ_{max} 251 nm, ε_{max} 200,000) of anthracene and the other two to the *primary bands* (λ_{max} 335 and 375 nm, ε_{max} 7500).[11] It appears evident that in **1**–**7** the intensity of the *short wavelength* band is very low with respect to anthracene. This difference can be explained by taking advantage of the X-ray crystallographic studies[4] of thermorubin (**1**) which show that in this molecule the *o*-hydroxyphenyl group attached to the 1,3-diketo group existing in the enolic form is oriented almost perpendicularly to the tetracyclic moiety. This is interpreted as due to the steric effect of the adjacent substituents and this fact brings about a non-planarity of the anthracene system, with a decreased absorption of the *short wavelength* band. So, leaving apart the differences in the absorption of the *primary bands*, which can be due to plain auxochromic effects of different substituents, our attention is devoted to the difference in the *short wavelength* band which is sensitive to conformation of the molecule as indicated above. The UV spectrum of **5**, which shows an enhancement of the intensity of the *short wavelength* band is in agreement with the loss of the 1,3-diketo moiety.

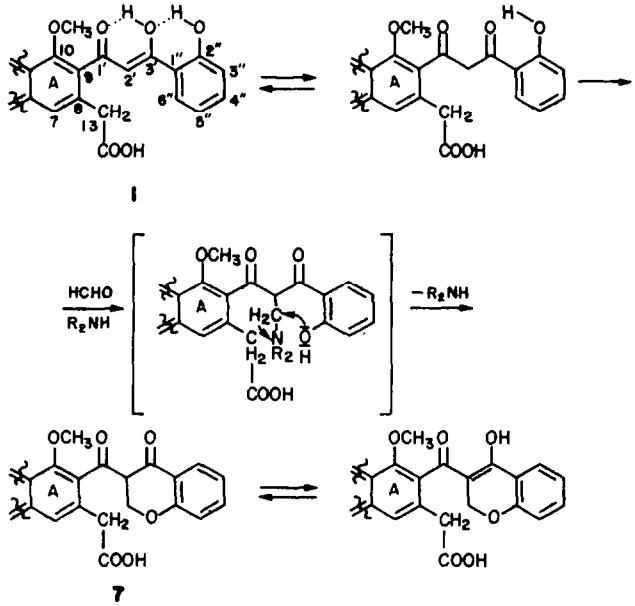
All the compounds obtained were tested for their antibacterial activity and found active in spite of the deep chemical modifications in respect to the original molecule. Structure–activity relationship will be published elsewhere.

EXPERIMENTAL

The reactions were monitored by TLC on Silicagel 60F-254 plates (Merck) developed with a mixture of $CHCl_3$–MeOH 9:1 (v/v) and the spots detected by UV light at both 254 and 360 nm. Solvents were evaporated under vacuum on a rotary evaporator at 40°.

UV spectra were run on a Beckman DK-2 spectrophotometer. IR spectra were recorded with a Perkin–Elmer model 580 spectrophotometer in nujol mull. ^{1}H-NMR spectra were recorded at 270 MHz with a Bruker WH-270 spectrometer in DMSO-d_6 or THF-d_8 soln with TMS as internal reference (δ = 0.00 ppm). LC/MS was done by a direct inlet system on a HP 5985 B instrument in both positive and negative ionization. Only positive ionization fragments are reported in Table 1. The instrument was equipped with a 10 cm RP-8 column eluted with CH_3CN–H_2O 75:25 or CH_3CN–THF–H_2O 70:10:20 or CH_3CN–DMSO–H_2O 70:10:20 according to the solubilities of the compound. The eluents were used as reactant gases for ionization.

Compound **2**. To a 35% soln of $MeNH_2$ in water (30 ml) previously cooled at −5° **1** (2.0 g, 3.3 mmol) was added with



Scheme 3.

vigorous stirring. The resulting dark-red mixture was stirred for 2 min then poured into ice, made acidic with conc HCl, extracted with EtOAc and dried over Na_2SO_4. Upon concentration yellow crystals separated that were collected, washed with a little EtOAc and dried under vacuum, yield 1.2 g.

Compound **3**. *From* **1**: Compound **1** (5.0 g, 8.3 mmol) was added to a 35% soln of $MeNH_2$ in water cooled at $-5°$ with stirring. The mixture was stirred for 5 min, poured into ice and worked up as reported above. The crude product was precipitated from EtOAc by addition of petroleum ether; TLC showed two main spots: one yellow (**2**, $R_f = 0.52$) and one light yellow (**3**, $R_f = 0.40$), the latter being predominant. Column chromatography (Silicagel 60 Merck 70–230 mesh loaded with 4.86% w/w potassium dihydrogen phosphate)[4] eluted with $CHCl_3$ containing increasing percentages of CH_3OH afforded 1.4 g of pure **3** and 0.25 g of pure **2**.

From **2**: Compound **2** (0.5 g, 0.8 mmol) was dissolved in 10 ml of 8% aqueous $MeNH_2$ and the resulting soln was stirred for 10 min at room temp after which TLC showed complete formation of **3**. The mixture was worked up as usual and pure **3** was obtained as yellow crystals from EtOAc, yield 0.3 g.

Compounds **4** *and* **5**. To a soln of **1** (5.0 g, 8.3 mmol) in THF (250 ml) potassium dihydrogen phosphate (5.0 g) was added. To this suspension a soln of $NaBH_4$ (5.0 g) in 95% EtOH (250 ml) was added in 15 min with stirring at room temp, while vigorous H_2 evolution took place. Stirring was continued for 10 hr after which TLC showed an orange spot ($R_f = 0.47$) and the disappearance of **1**. The mixture was then transferred into a separatory funnel, water (1 l) and EtOAc (500 ml) were added, followed by 10% HCl in order to destroy the excess $NaBH_4$ and make the pH acidic. The content of the funnel was vigorously shaken, the organic phase was separated and the aqueous layer was extracted again with EtOAc (500 ml). The combined organic phases were washed with water to neutrality and dried over Na_2SO_4. TLC showed two predominant orange spots ($R_f = 0.47$ and 0.49). After concentration to a small volume, addition of petroleum ether gave a mixture of the two compounds, which were separated by column chromatography (Silicagel 60 Merck) eluted with CH_2Cl_2 containing from 3 to 10% of MeOH. The fractions were checked by TLC and combined. Compound **4** was recrystallized from MeOH (red crystals, 1.5 g); **5** was precipitated as an orange powder from EtOAc–petroleum ether (1.1 g).

Compound **6**. To a 33% soln of $MeNH_2$ in abs EtOH (25 ml) 0.5 g of the free diacid obtained by treatment of **1** (0.6 g, 1 mmol) with 0.1 N NaOH followed by acidification and extraction with EtOAc was added at room temp with stirring. After stirring the mixture for 10 min a soln was obtained, that was poured into ice–water and then made acidic with conc HCl. A yellow ppt separated which, after a few min, turned red and gummy. The supernatant was discarded, and the product was washed twice with water and then triturated with EtOAc. Upon filtration crude **6** was recovered. Pure **6** (0.24 g) was obtained as red crystals from MeOH.

Compound **7**. To a mixture of 0.33 ml of 40% aqueous formaldehyde and equimolar amounts of selected amines in THF (200 ml), **1** (1.8 g, 3 mmol) was added and the resulting soln was stirred at room temp for 30 min after which the reaction was complete. The mixture was concentrated to a small volume, diluted with water, acidified with a few drops of conc HCl and extracted with EtOAc (2 × 200 ml). The organic layer was separated, washed with water to neutrality and dried over Na_2SO_4. Upon concentration 1.27 g of crude was obtained that was purified by column chromatography (Silicagel 60 Merck), eluting with a mixture 99:1 (v/v) of CH_2Cl_2 and MeOH. Fractions containing **7** (TLC) were evaporated giving 0.64 g of an orange crystalline solvate with CH_2Cl_2.

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