extracted with either, dried ($MgSO_4$), and evaporated to dryness, resulting in 256 mg of yellow oil whose ^1H NMR spectrum was consistent with the analysis reported in the Discussion section. Separation of individual components by LC[8] followed by ^2H NMR analysis gave the results also reported in the earlier part of this paper. When the photoreaction was carried out at 73 °C, the results were identical, within experimental error.

Direct Irradiation of 9-OMs. A solution[8] of 185 mg (0.683 mmol) of a mixture of 40% **9**-OMs and 60% **2**-OMs in 5.25 mL of dry acetonitrile was deoxygenated and irradiated (254 nm) as above for 2.75 h. The reaction mixture was worked up and analyzed as above, giving the product ratios described in the earlier section.

Direct Irradiation of 10-OMs. A 83:17 mixture of **10**-OMs–**11**-OMs (110 mg, 0.38 mmol) in 1.1 mL of dry acetonitrile was placed in a 5-mm quartz NMR tube and deoxygenated as above. The tube was sealed and irradiated (254 nm) in the "Photoprep" apparatus[8] for 15.25 h. Workup as above followed by LC[8] analysis and separation gave the product ratio described in the earlier section, and ^2H NMR analysis gave the deuterium ratios described above.

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Thermorubin. 1. Structure Studies

Francis Johnson,* Bani Chandra, Charles R. Iden, Prakash Naiksatam, Robert Kahen, Yoshi Okaya,* and Shue-Yih Lin

Contribution from the Departments of Pharmacological Sciences and Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794. *Received June 7, 1979*

Abstract: The structure of thermorubin, thought previously to be 5,7-dihydroxy-3,6-dimethoxy-2-[1′,3′-dihydroxy-2′-methoxycarbonyl-9′-xanthon-4′-yl)methyl]anthracene, is shown by physical and chemical evidence to be incorrect. A new structure, namely, 12-hydroxy-10,11-dimethoxy-3-methoxycarbonyl-8-carboxymethyl-9-[1′-oxo-3′-hydroxy-3′-(2″-hydroxyphenyl)-prop-2′-enyl]-1*H*-2-oxanaphthacen-1-one, has been determined largely by an X-ray diffraction analysis, and this accounts for all of the previously observed chemical degradation results. The structure of trimethylthermorubin, obtained by simple methylation of thermorubin, is also elucidated.

I. Introduction

In 1964 Craveri, Coronelli, Pagani, and Sensi[1] described a new thermophilic actinomycete, *Thermoactinomyces antibioticus*, which in submerged culture produced a novel antibiotic substance. This material, termed thermorubin, proved to be very active against Gram-positive bacteria, less active against Gram-negative bacteria, and virtually inactive against yeasts and filamentous fungi. A group[2] from the same organization subsequently investigated the mechanism of antibiotic action and found that thermorubin is bacteriostatic and inhibits protein synthesis at the level of translation, but that DNA and RNA syntheses are unaffected. They reported that, in vitro, thermorubin inhibits protein synthesis directed by natural messenger RNA, but not the synthesis of poly-Phe directed by the synthetic messenger poly-U. The binding of the initiator of protein synthesis, fmet-$tRNA^{met}$, to 70S ribosomes is inhibited, but the reaction of puromycin (an analogue of aminoacyl-tRNA) with initiator already bound to the ribosome is not impaired. In a related study Wishnia and Lin[3] have found, however, that thermorubin does not inhibit the association of this initiator with the 30S ribosomal subunit. Thus thermorubin becomes the first example of an inhibitor which prevents initiator attachment *only* when the 30S and 50S subunits are in association. The latter investigations have also demonstrated that thermorubin very strongly inhibits dissociation of 70S ribosomes. One possible mechanism of bacteriostatic action might be the prevention of the return of the 30S subunits to the common pool. Thermorubin shows no cross-resistance with other known antibiotics and this, together with its seemingly low toxicity (LD_{50} = 300 mg/kg intraperitoneally in mice), would appear to make it a likely candidate for development as an agent for therapeutic use in man. A drawback is that it has very low solubility in physiological fluids, and its activity in vitro is diminished in the presence of animal serum. Nevertheless, it affords 100% protection to mice against *Staphylococcus aureus* infections when administered intraperitoneally for 3 days at the level of 3 mg/kg body weight. The reason for the selective action of thermorubin against procaryotic cells is unknown at this time.

These pharmacological properties form the basis for our continued interest in the structure and in the chemical modification of this antibiotic.

Table I. Comparison of NMR Data for the Four Coupled Protons

compd in C_6D_6	hydrogen resonance position,[a] δ			
	$H_{5'}$	$H_{6'}$	$H_{7'}$	$H_{8'}$
2[b]	6.96	7.18	6.68	7.79
3	6.80	7.04	7.04	8.12

[a] Each of these absorptions is a complex but fairly symmetrical multiplet; assignment of the positions was made on the basis of decoupling experiments already described.[5] [b] A pictorial view of this region of the NMR spectrum has already been published.[5]

II. Previous Structure Studies

Initially, when fermentation broth of *Thermoactinomyces antibioticus* was extracted with organic solvents, the crude material was thought to comprise largely a single product (70–80%). This, after recrystallization from chloroform, afforded the chloroform solvate of thermorubin as a bright red powder. The early studies[1] claimed that thermorubin (a) is optically active, (b) has a molecular weight of 432, (c) has the empirical formula $C_{22}H_{18}O_8$, (d) contains two weakly acidic groups, and (e) has two methoxy groups and an *o*-hydroxyquinone moiety (infrared data). Proof of the presence of the latter group was adduced by the observation that thermorubin was decolorized by a variety of reducing agents. In addition, it was claimed that thermorubin gives a diacetyl derivative when treated with acetic anhydride/pyridine, that it reacts[4] with diazomethane to give a trimethyl ether, and that, on fusion with sodium hydroxide, it yields[4] salicylic acid.

(1) Craveri, R.; Coronelli, C.; Pagani, H.; Sensi, P. *Clin. Med.* **1964**, *71*, 511–522.
(2) Pirali, G.; Somma, S.; Lancini, G. C.; Sala, F. *Biochim. Biophys. Acta* **1974**, *366*, 310–318.
(3) Wishnia, A.; Lin, Fwu-Lai (Department of Chemistry, State University of New York at Stony Brook), private communication.
(4) Maggi, N. (Gruppo Lepetit Spa, Milan, Italy), private communication.

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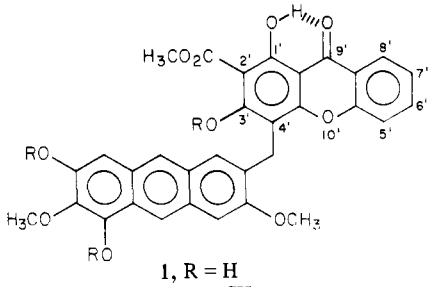
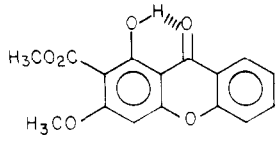
Against this background, Moppett, Dix, Johnson, and Coronelli[5] undertook a study of this antibiotic and in 1971 assigned to it structure **1** ($C_{32}H_{24}O_{10}$). This assignment was made on the basis of an examination of the physical data of thermorubin and, more importantly, of the trimethyl derivative, designated as **2**, together with a comparison of certain of these data with those derived from **3**, a compound synthesized[5] for the purpose.

Although the case for structure **1** appeared almost watertight, one of the present authors, who at the time was concerned mainly with the synthesis of **3**, retained some doubts. Chief among these



1, R = H
2, R = CH_3



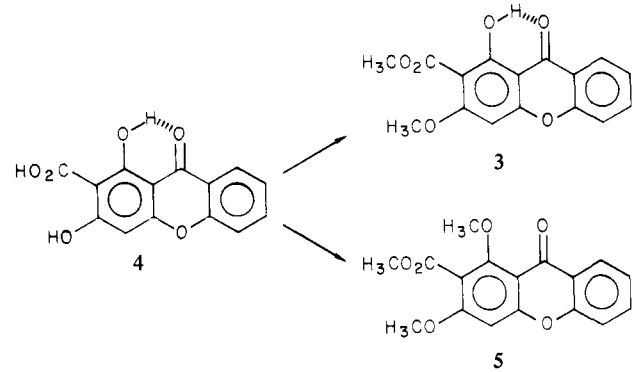
3

was the simple fact that **2** has a deep orange-red color, whereas **3** and known polymethoxylated anthracenes are pale yellow in color. Neither did it seem possible that such color could be due to an intramolecular charge-transfer complex between the xanthone and anthracene moieties because of obvious restrictions in geometry. Furthermore, although the overall pattern is similar and a reasonable congruency should be expected, the positions of the complex multiplets in the ^1H NMR spectrum of **3**, assigned to the four coupled aromatic protons, did not agree well with those observed for the corresponding protons in **2** (see Table I). The same could be said when a comparison is made of the 1600–1800-cm^{-1} region of the solution ($CHCl_3$) infrared spectra of these two compounds. The spectrum of **2** shows bands at 1603, 1635, and 1740 cm^{-1}, whereas that of **3** has bands at 1612, 1648, and 1725 cm^{-1}. Much closer spectral agreement would be expected if **3** is an integral unit in **2**. Other discrepancies began to emerge rapidly as soon as a reinvestigation of the problem was initiated. Not only was the structure assigned[5] to thermorubin thrown into serious doubt, but most of our results, which are presented below, were found to be in conflict with the initial Italian paper.[1]

III. Recent Chemical and Physicochemical Studies

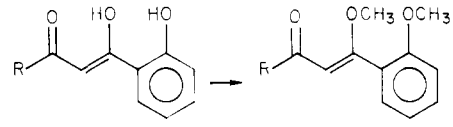
Crude thermorubin, as supplied by Gruppo Lepetit Spa (Italy), is an orange-brown or buff-colored powder which resisted all attempts to purify it by direct recrystallization. Purification was finally achieved[6] by chromatography over silica gel loaded with 2–4% potassium dihydrogen phosphate followed by recrystallization from chloroform. Thermorubin isolated in this way is a red, microcrystalline chloroform solvate which is stable in air. However, in solution it must be kept under nitrogen to prevent a fairly fast aerial oxidation. Its solutions also appear to be light sensitive, but deterioration via this route is not rapid.

Because all initial attempts to obtain a crystal of thermorubin sufficiently large for X-ray diffraction studies failed, we turned to analyses of chemical and other physical data. Contrary to previous reports,[1] thermorubin proved to be easily soluble in base and liberates carbon dioxide from sodium bicarbonate solution, indicative of the presence of a carboxylic acid. A study[3] of the pH titration spectra in the ultraviolet region revealed the presence of three acidic groups having pK_a values of 4.7, 7.0, and 9.0 characteristic, respectively, of a carboxylic acid, a phenol, and possibly an enolic system. The presence of an additional hydroxyl group was evident from the ^1H NMR spectrum of trimethylthermorubin, which shows a deuterium-exchangeable proton at δ 13.27. That this is probably a highly chelated, nonacidic hydrogen atom is evident from the fact that extended treatment (10 days) of trimethylthermorubin with diazomethane does not lead to further methylation. That this hydroxyl group is *not* located in a position similar to that of the OH in **3** is indicated by the fact that, when the latter compound or its precursor[7] **4** is treated with diazomethane for 48 h, it yields the permethylated product **5**. The *complete absence* of a xanthone system in thermorubin



4 **3** **5**

became evident when it was found that oxidation of its trimethyl derivative with cold potassium permanganate solution *leads to methyl 2-methoxybenzoate* and traces of other products. Reexamination of the action of alkali on thermorubin revealed that salicylic acid is produced even under relatively mild hydrolysis conditions. The fusion reaction conditions[4] described earlier are entirely unnecessary, and thus the interpretation[5] of the production of salicylic acid as coming from a Haller–Bauer cleavage of a xanthone had to be abandoned. The degradations described above lead to the inescapable conclusion that thermorubin contains a tautomerically enolizable system, possibly a 1,3-diketone such as is depicted in **6**, that on methylation gives rise to **7**. This would



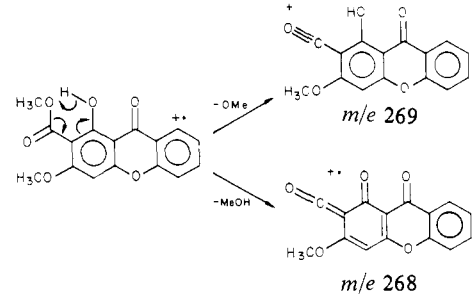
6 **7**

also account for the fact that methylation of thermorubin by diazomethane gives a mixture of products (trimethylthermorubin is dominant) and not a single substance as could be expected on

(5) Moppett, C. E.; Dix, D. T.; Johnson, F.; Coronelli, C. *J. Am. Chem. Soc.* **1972**, *94*, 3269–3272.

(6) We should like to thank Dr. R. Cricchio, who developed this method of purification while working in the authors' laboratory.

(7) The proof of structure for xanthones **3**, **4**, and **5** lies in the metastable transitions (determined by the defocusing technique) in the mass spectrum of **3**. The spectrum shows ions at *m/e* 268 and 269 corresponding to the decomposition pattern shown below. The loss of methanol in particular leaves

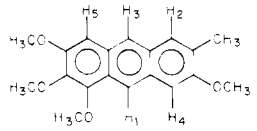
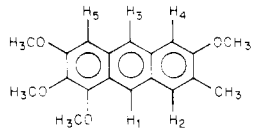


m/e 269

m/e 268

little doubt that the carbomethoxy is adjacent to a hydroxy group. In addition, the known difficulty observed in the methylation of the 1-hydroxyxanthones, seen in the conversion of **3** to **5**, indicates that the hydroxyl in **3** is at the 1 position; therefore, by inference carboxylation of 1,3-dihydroxyxanthone occurs at the 2 position.

Table II

	hydrogen resonance positions,[a] δ				
	H_1	H_2	H_3	H_4	H_5
trimethylthermorubin	7.86	7.81	8.26	7.42	6.25
 8	7.89	7.49	8.45	7.07	6.83
 9	7.73	7.43	8.14	6.80	6.69

[a] Assignments were made for 8 and 9 using methods (double and triple irradiation effects and observations of NOE) described[5] previously.

the basis of structure **1**. The methylation of most enolizable 1,3-diketones with diazomethane is known to give at least two monomethyl derivatives.

Structure **1** became completely untenable when it was found that the ^1H NMR resonances of either anthracene[8] **8** or **9** did not correspond well with those attributed[5] to the corresponding protons in **2** (see Table II). Perhaps the most significant point about these data is the disparity that exists in the positions of the resonances assigned to the protons ortho to the methoxy groups. In the model compounds the absorptions for these hydrogen atoms, H_4 and H_5, are between 0.3 and 0.6 ppm different in position from the corresponding proton absorptions attributed to the anthracene moiety of trimethylthermorubin. In particular, the resonance position of H_5 in **8** could be expected to approximate very closely the corresponding line[5] in trimethylthermorubin, considering that it is farthest away from any electronic influence that the supposed xanthone moiety would have. That little relationship exists between thermorubin and the postulated structure **1** is also evident from the fact that the ultraviolet spectrum (either at low or high concentrations) of an equimolar mixture of the xanthone **3** and either of the anthracenes **8** or **9** did not resemble the spectrum of trimethylthermorubin. The UV spectra of the mixtures were found to be no more complicated than the simple addition of the individual spectra of the constituent compounds. The intense peak at 325 nm (ε 50 800) present in the spectrum of trimethylthermorubin is completely absent from those of the mixtures. In addition, the spectra of the latter were completely transparent above 350 nm, whereas peaks of moderate intensity (ε 6000–7000) are present in the UV spectrum of trimethylthermorubin at 403 and 424 nm.

At this point, a more extensive investigation of the spectral data of thermorubin and its trimethyl derivative was undertaken. However, despite substantial efforts to correlate the ^{13}C and ^1H NMR spectra of these compounds with known systems, little new useful information was obtained except that the ^{13}C spectra of thermorubin (a) confirmed that thermorubin is a C_{32} compound having three methoxy groups (lines at 52.69, 63.79, and 64.05 Hz which appear as quartets in an off-resonance experiment), and (b) showed the presence of three carbonyl groups (lines at 166.0, 171.6, and 187.1 Hz which remain as singlets in an off-resonance experiment) rather than two as previously found.[5] Not the least of the problems associated with obtaining these spectra is the fact that thermorubin has limited solubility in most of the common organic solvents and when in solution easily tautomerizes to a mixture of various enolic and keto forms. Both compounding our difficulties and confounding our efforts at structure determination were the mass-spectral data, which could not be rationalized until the X-ray diffraction data (vide infra) had revealed the true structure of thermorubin. In short, it seems that *neither thermorubin nor any of its derivatives*[9] *volatilizes in the mass spectrometer until an intermolecular transfer of a methyl group (or a methylene) has taken place*, and in this respect they resemble the vinca alkaloids[10] which show ions at both *m/e* M + 14 and M + 28. Thermorubin consistently shows an ion at *m/e* 614, whereas, as will be shown later, the true molecular weight is 600. Unfortunately, a molecular weight of 614 conforms to the basic structure of a pentadecaketide after allowing for nonskeletal carbons (three methoxy groups), whereas a molecular weight of 600 does not. The highest *m/e* exhibited by trimethylthermorubin is 656 rather than the value 610 reported[5] previously, and from which it was concluded erroneously that thermorubin had a molecular weight of 568. The 656 peak observed for trimethylthermorubin is, of course, not the molecular ion (which is also seen at *m/e* 642) but the $[M + 14]^+$ peak in keeping with what was said above.

IV. X-ray Crystallographic Studies

A. Experimental Section. With the failure of the usual physicochemical methods to provide useful information we renewed our earlier efforts to obtain a crystal of thermorubin sufficiently large for X-ray diffraction analysis. After almost innumerable attempts, it was found that, when a saturated solution of thermorubin in chloroform was added slowly to a boiling solution of chloroform in a deep, narrow tube immersed in an oil bath (so that the oil level was always just below that of the chloroform) and maintained at a temperature such that a slow distillation of solvent takes place, a supersaturated solution was obtained. When this was allowed to cool under nitrogen over a 4-h period, a mass of very small, brilliantly red, monoclinic blades of thermorubin chloroform solvate was formed in which were located several crystals that were judged large enough for an X-ray diffraction study.

The crystal selected for data collection (0.25 × 0.02 × 0.15 mm) was mounted on a CAD4 A diffractometer driven by a PDP-8 computer under the control of a resource-sharing PDP-11/45 computer.[11] Twenty relatively strong reflections were found and centered on the diffractometer using Cu Kα radiation. The unit cell dimensions, obtained at room temperature by an indexing program followed by a least-squares fitting of the reflection data, are $a = 7.122$ (2) Å, $b = 41.78$ (1) Å, $c = 10.875$ (3) Å, $\beta = 96.72$ (2)°, and $V = 3214$ (2) Å^3. The space group of the crystal was unambiguously determined to be $P2_1/a$ by the following systematic absences: $h0l$ for or h = odd and $0k0$ for k = odd. There are four molecules in the unit cell each associated with a chloroform molecule. Intensity data were collected up to $\theta = 65°$ using a θ–2θ scan technique. A total of 5700 reflections were recorded. There were 1842 reflections having intensities greater than 1σ, and 1109 were greater than 3σ. The limited number of meaningful reflections was due to the small size of the available specimen.

B. Determination of the Structure and Molecular Configuration. The structure was solved[12] by the direct method (MULTAN[13]) using

(8) (a) The synthesis of **8** was accomplished by a new method. A report describing its generality and scope for the synthesis of anthracenes will be the subject of a separate publication. (b) The synthesis of **9** is an example of a general method for the synthesis of anthracenes and anthraquinones that was published recently: Kim, K. S.; Spatz, M. W.; Johnson, F. *Tetrahedron Lett.* **1979**, 331–334.

(9) Other compounds derived from thermorubin that show the presence of an $[M + 14]^+$ ion in their mass spectra include a dehydration product and a transformation product containing four acetoxy groups obtained by the action of acetic anhydride–pyridine on thermorubin. The structure of these latter two compounds will be discussed in a subsequent paper.

(10) Bommer, P.; McMurray, W.; Biemann, K. *J. Am. Chem. Soc.* **1964**, *86*, 1439–1440. Biemann, K. *Lloydia* **1964**, *27*, 397–405.

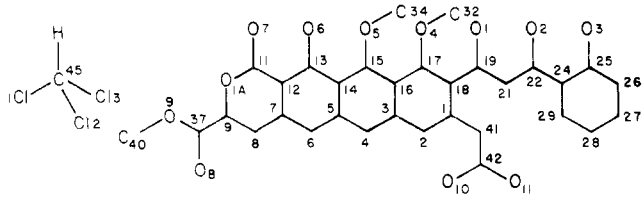
(11) Okaya, Y. In "Computing in Crystallography", International Union of Crystallography; Schenk, H., Ed.; Delft University Press: Delft, 1978; p 153.

(12) All calculations were made on a PDP-11/45 computer using the Enraf-Nonius structure determination package.

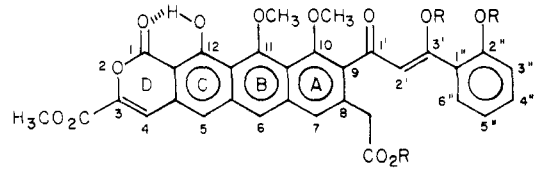
(13) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A* **1971**, *27*, 368–376.

about 300 E factors above 1.97, and as usual for $P2_1/a$ the phase set with the best figure of merit yielded a good trial structure of 35 atoms. The trial structure had a flat, linear, tetracyclic skeleton similar to naphthacene with several substituents. Since at this stage the structure of the molecule was uncertain, all of the atoms except the chlorines of the chloroform molecule were assigned as carbon atoms, despite the fact that substantial chemical evidence indicated that methoxy, keto, phenol, and acid groups were present. After several cycles of least-squares refinement with individual isotropic thermal parameters, the unweighted residual $R_{uw} = \sum|F_o - F_c|/\sum|F_o|$ was 30% with many atoms showing negative temperature factors. The subsequent difference Fourier syntheses revealed nine more atoms which further reduced R_{uw} to 25%. At this point, those atoms with negative temperature factors were reassigned as oxygen, which conforms to the chemical evidence presented earlier. However, in order to avoid drastic changes in the nature of the ring system, an atom in one of the end rings which exhibited a negative temperature factor was kept as carbon. The reassignments improved the R_{uw} factor to 20%. Four more atoms were located by a further difference Fourier synthesis. At this stage a total of 48 atoms were refined to give $R_{uw} = 15\%$ using isotropic thermal parameters. The temperature factor of the carbon atom in the end ring, mentioned earlier, remained negative throughout these refinements. The establishment of the end ring as a lactonic structure by changing this carbon atom to an oxygen atom lowered R_{uw} to 13%.

The configuration of the 48 atoms obtained thus far is shown as structure **10**. It contains very reasonable bond distances and



10



11, R = H
12, R = CH_3

bond angles, although there was considerable uncertainty associated with atoms 21–29, and this is discussed later. One of the substituents [C(41), C(42), O(10), O(11)] at one end of the tetracyclic structure turned out to be an acetic acid residue, explaining the pK_a value of 4.7. Ortho to this substituent is a 1,3-diketone (in its enolic form) bearing an o-hydroxyphenyl group at its terminus. This keto–enolic system is oriented almost perpendicularly to the tetracyclic moiety because of the steric effects of the adjacent substituents. The major portion of this side chain (atoms 21–29) showed remarkably high thermal vibration possibly due to the relatively unrestricted nature of the assembly and the mode of crystal packing. In an attempt to reduce these thermal effects, X-ray crystallographic data were gathered at low temperatures.

C. Low-Temperature Study. A similar set of data was collected at about −150 °C using a cold-stream cooling system with liquid nitrogen. The cell constants obtained are $a = 7.032$ (3) Å, $b = 41.59$ (2) Å, $c = 10.691$ (7) Å, and $\beta = 96.19$ (4)°. Although theoretically this should result in obtaining additional intensity data with higher σ's (especially at higher θ values), most of the crystals, which were very small to begin with, cracked under the sudden temperature changes. Nevertheless, this data did yield 1378 reflections with intensities greater than 3σ.

When the coordinates obtained from the room-temperature study were used, the isotropic least-squares refinements of the low-temperature data converged to $R_{uw} = 11.3\%$ ($R_w = 12.2\%$).

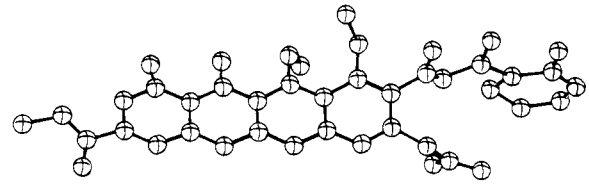


Figure 1. ORTEP diagram of thermorubin (face view).

The whole molecule definitely shows lower temperature factors with better established coordinates at −150 °C. However, the positional and thermal data for most of the salicyloyl side chain still deviate substantially from the normal values. As explained before, it is possible that most of this side chain may sit in relatively vacant space in the crystal with enough freedom to vibrate or to occupy several subsidiary sites. Thus cooling may not be able to freeze the constituent atoms into a definite position. Despite the fact that this group gives rather unsatisfactory diffraction data, the chemical and physicochemical work described previously leaves no doubt as to its character and its mode of attachment to the tetracyclic framework.

In the last stage of the data analysis, all the nonhydrogen atoms were assigned anisotropic thermal parameters, and most of these, except those of the larger side chain, were subjected to a final refinement. The unweighted R factor converged at 10.5% ($R_w = 11.2\%$). Sixteen hydrogen atoms in the condensed part of the structure were easily located by difference Fourier maps. These were included in the structure-factor calculations but were not refined. Those which were difficult to locate were omitted from the calculations. The final R_{uw} factor obtained from 1378 reflections ($>3\sigma$) was 9.3% with $R_w = 9.5\%$. A difference electron-density map calculated at this stage does not show any residual density higher than 0.6 e Å^{-3}; hence the refinement is considered complete. The atomic coordinates obtained for the low-temperature data are listed in Table III, and the bond distances and angles calculated[12] using these data are given in Table IV.

The function minimized in the least-squares refinements is $\sum w(|F_o| - |F_c|)^2$ with $w^{-1} = \sigma_c F_o^2 + (0.03F_o^2)^2$, where σ_c is the deviation based on counting statistics.

D. Discussion. The numbering of the carbon and oxygen atoms used in the discussion of the X-ray diffraction data is shown in **10**, and the chemical-formula numbering is given in **11**. The ORTEP diagram for thermorubin is depicted in Figure 1.

As can be seen from the table of bond distances, the aromatic bonds in the tetracyclic moiety vary in length between 1.31 and 1.44 Å with an average value of 1.40 Å. This average value is also seen for the aromatic bonds of the o-hydroxyphenyl group, but because of the higher thermal vibrations they have much wider variations. The C(11)–O(7) bond length is 1.23 Å, identifying this as a carbonyl group. At the other end of the molecule the C–O bond distances of 1.35 [C(19)–O(1)] and 1.44 Å [C(22)–O(2)], associated with the three aliphatic carbons of the side chains, are somewhat longer than those seen for normal carbonyl groups but are in agreement with the enolic form shown in **11**. Nevertheless, not too much confidence can be placed in these values because of the large thermal vibrations involved, and if it were not for the chemical evidence this conclusion would be suspect because the bond distances observed for C(19)–C(21) and C(21)–C(22) (1.34 and 1.53 Å, respectively) are the reverse of what might be expected for single and double bonds of this type. It may well be that a moderate percentage of the other cis keto–enol is also present. Nevertheless, the planar character of the array O(1)–C(19)–C(21)–C(22)–O(2) leaves little doubt that the system is a keto–enol rather than a 1,3-dione.

Some interesting effects associated with the bond angles can be observed. The plane of this enolic dione [C(19)–O(1)–C(21)–C(22)–O(2)] makes a dihedral angle of 83° to the adjacent aromatic plane [C(1)–C(2)–C(3)–C(16)–C(17)–C(18)], as noted earlier, in order to minimize steric interactions with the adjacent methoxy and acetic acid groups. In the lactone ring the C(9)–O(1A)–C(11) bond angle opens up to 120°, whereas normally

Table III. Atomic Coordinates with Esd's

atom	x	y	z
Cl1	0.5256 (6)	0.1841 (1)	0.3259 (4)
Cl2	0.1108 (6)	0.1831 (1)	0.2885 (4)
Cl3	0.3093 (7)	0.2422 (1)	0.3597 (4)
O1A	0.667 (7)	0.1870 (3)	0.8013 (10)
O1	0.549 (3)	0.0468 (5)	0.7090 (21)
O2	0.529 (4)	0.0075 (6)	0.3607 (24)
O3	0.566 (4)	0.0681 (7)	0.3351 (27)
O4	0.275 (2)	0.1057 (3)	0.6028 (11)
O5	0.138 (1)	0.1653 (3)	0.6046 (10)
O6	0.070 (2)	0.2250 (3)	0.6137 (9)
O7	0.561 (2)	0.2123 (3)	0.6298 (10)
O8	0.823 (2)	0.1508 (3)	0.0911 (11)
O9	0.775 (2)	0.1328 (3)	0.8943 (11)
O10	0.054 (2)	0.0372 (3)	0.9654 (12)
O11	0.250 (2)	−0.0010 (3)	0.0476 (11)
C1	0.335 (2)	0.0804 (4)	0.929 (2)
C2	0.340 (2)	0.1075 (5)	−0.008 (2)
C3	0.294 (2)	0.1371 (4)	0.928 (2)
C4	0.297 (2)	0.1663 (5)	−0.011 (2)
C5	0.256 (2)	0.1960 (4)	0.929 (2)
C6	0.275 (2)	0.2366 (4)	−0.010 (1)
C7	0.739 (2)	0.2442 (4)	0.932 (2)
C8	0.775 (2)	0.215 (5)	−0.009 (2)
C9	0.737 (2)	0.1871 (4)	0.931 (2)
C11	0.632 (2)	0.2158 (4)	0.739 (1)
C12	0.669 (2)	0.2454 (4)	0.801 (2)
C13	0.143 (2)	0.2250 (4)	0.734 (2)
C14	0.193 (2)	0.1960 (4)	0.796 (1)
C15	0.196 (2)	0.1665 (4)	0.733 (1)
C16	0.257 (2)	0.1379 (4)	0.795 (2)
C17	0.282 (2)	0.1080 (4)	0.732 (2)
C18	0.324 (2)	0.0797 (4)	0.796 (2)
C19	0.363 (4)	0.0481 (6)	0.731 (2)
C21	0.209 (5)	0.0298 (9)	0.698 (3)
C22	0.276 (5)	−0.0037 (9)	0.665 (3)
C24	0.834 (5)	0.0343 (8)	0.330 (3)
C25	0.757 (5)	0.0687 (8)	0.328 (3)
C26	0.893 (6)	0.0928 (10)	0.312 (4)
C27	1.041 (6)	0.0831 (10)	0.309 (4)
C28	0.112 (7)	0.0495 (11)	0.302 (4)
C29	0.996 (7)	0.0186 (10)	0.334 (4)
C32	0.433 (2)	0.1203 (5)	0.546 (2)
C34	0.942 (2)	0.1523 (5)	0.579 (2)
C37	0.781 (2)	0.1546 (4)	−0.018 (2)
C40	0.818 (2)	0.0995 (4)	0.943 (2)
C41	0.385 (3)	0.0501 (4)	0.006 (2)
C42	0.213 (3)	0.0268 (4)	0.005 (2)
C45	0.307 (2)	0.2016 (4)	0.380 (1)

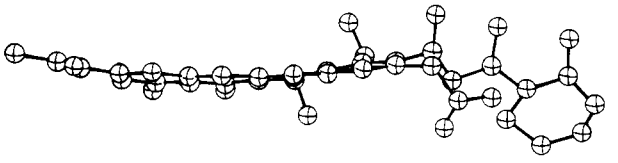


Figure 2. ORTEP diagram of thermorubin (edge view).

a value of ~100° is seen for this angle in a simple ester.

The overall shape of the tetracyclic skeleton of thermorubin is planar; however, the equations of various planes indicate that it exists rather in a slightly curved conformation as noted in the second ORTEP diagram (Figure 2). Finally, as might be expected for a carboxylic acid, a pair of molecules associate centrosymmetrically in the crystal with an O–H···O hydrogen bond distance of 2.61 Å.

The assignment of structure **12** (R = CH_3) to trimethylthermorubin follows naturally from the fact that trimethylthermorubin is not a carboxylic acid (cf. thermorubin itself) and as noted above gives methyl 2-methoxybenzoate on permanganate oxidation.

V. Note of the Biogenesis

The structure of thermorubin is quite remarkable from both a chemical and biological point of view. Not only is the molecule

Table IV. Thermorubin

A. Bond Distances (Å)

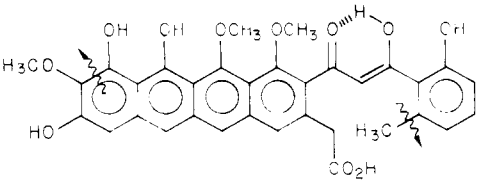
C1–C2	1.31 (2)	C11–C12	1.41 (2)	C22–O2	1.44 (3)
C1–C18	1.42 (2)	C11–O7	1.23 (1)	C24–C25	1.53 (4)
C1–C41	1.52 (2)	C12–C13	1.43 (2)	C24–C29	1.31 (4)
C2–C3	1.43 (2)	C13–C14	1.40 (2)	C25–C26	1.41 (4)
C3–C4	1.38 (2)	C13–O6	1.33 (1)	C25–O3	1.35 (3)
C3–C16	1.42 (2)	C14–C15	1.40 (2)	C26–C27	1.12 (8)
C4–C5	1.41 (2)	C15–C16	1.41 (2)	C27–C28	1.49 (6)
C5–C6	1.43 (2)	C15–O5	1.39 (1)	C28–C29	1.58 (6)
C5–C14	1.44 (2)	C16–C17	1.44 (2)	C32–O4	1.45 (1)
C6–C7	1.38 (2)	C17–C18	1.38 (2)	C34–O5	1.48 (1)
C7–C8	1.37 (2)	C17–O4	1.38 (1)	C37–O8	1.18 (1)
C7–C12	1.44 (2)	C18–C19	1.53 (2)	C37–O9	1.30 (2)
C8–C9	1.35 (2)	C19–O1	1.35 (2)	C40–O9	1.50 (1)
C9–O1A	1.42 (1)	C19–C21	1.34 (3)	C41–C42	1.55 (2)
C9–C37	1.48 (2)	C21–C22	1.53 (4)	C42–O10	1.23 (2)
O1A–C11	1.38 (1)	C22–C24	1.50 (4)	C42–O11	1.26 (2)

B. Bond Angles (Deg)

C2–C1–C18	122 (2)	C12–C11–O7	126 (1)
C2–C1–C41	117 (2)	C7–C12–C11	117 (1)
C18–C1–C41	121 (2)	C7–C12–C13	122 (1)
C1–C2–C3	121 (2)	C11–C12–C13	121 (1)
C2–C3–C4	123 (2)	C12–C13–C14	120 (1)
C2–C3–C16	120 (1)	C12–C13–O6	120 (1)
C4–C3–C16	116 (2)	C14–C13–O6	120 (1)
C3–C4–C5	125 (1)	C5–C14–C13	120 (1)
C4–C5–C6	125 (1)	C4–C14–C15	117 (1)
C4–C5–C14	118 (1)	C13–C14–C15	123 (1)
C6–C5–C14	117 (1)	C14–C15–C16	123 (1)
C5–C6–C7	125 (1)	C14–C15–O5	119 (1)
C6–C7–C8	124 (1)	C16–C15–O5	118 (1)
C6–C7–C12	116 (1)	C3–C16–C15	120 (1)
C8–C7–C12	120 (1)	C3–C16–C17	116 (1)
C7–C8–C9	122 (2)	C15–C16–C17	124 (1)
C8–C9–O1A	120 (2)	C16–C17–C18	123 (1)
C8–C9–C37	127 (2)	C16–C17–O4	122 (1)
C37–C9–O1A	113 (2)	C18–C17–O4	115 (1)
C9–O1A–C11	119 (1)	C1–C18–C17	118 (1)
O1A–C11–C12	122 (1)	C1–C18–C19	118 (2)
O1A–C11–O7	113 (1)	C17–C18–C19	124 (2)
C18–C19–O1	110 (2)	C26–C27–C28	131 (7)
C18–C19–C21	115 (2)	C27–C28–C29	125 (5)
C21–C19–O1	134 (2)	C24–C29–C28	93 (5)
C19–C21–C22	109 (2)	C17–O4–C32	116 (1)
C21–C22–O2	118 (3)	C15–O5–C34	111 (1)
C21–C22–C24	127 (3)	C9–C37–O8	120 (2)
C24–C22–O2	115 (4)	C9–C37–O9	113 (2)
C22–C24–C25	128 (4)	O8–C37–O9	127 (2)
C22–C24–C29	92 (3)	C37–O9–C40	114 (1)
C25–C24–C29	140 (4)	C1–C41–C42	115 (1)
C24–C25–C26	115 (3)	C41–C42–O10	118 (1)
C24–C25–O3	110 (4)	C41–C42–O11	116 (1)
C25–C25–O3	135 (5)	O10–C42–O11	126 (2)
C25–C26–C27	113 (6)		

of a unique type, but to find such high biological activity (the level of activity and spectrum of action are roughly equivalent to those of penicillin G) associated with a naturally occurring, relatively nontoxic molecule *lacking optical activity* is comparatively rare.

The biogenesis of this substance presents some interesting questions. It seems likely that the bulk, if not all, of the molecule is derived from a polyketide condensation. To arrive at **11** would then require a pentadecaketide precursor followed by metabolic degradation of an intermediate molecule such as **13** in which ring



13

D is cleaved (Woodward fission?) as shown to give a carboxylic acid and a methyl pyruvate residue. Lactonization of these two

groups would give ring D of **11**. The other oxidative process needed is an oxidative decarboxylation of the 6″-methyl group of the phenyl ring.

Alternatively, it could be considered that thermorubin is assembled from an undecaketide precursor and salicylic acid, the latter arising via the shikimate pathway.[14] Such a pathway would be interesting because the latter biogenetic route is found[15] most often in the realm of higher plants and very rarely in fungi.

A third biosynthetic possibility is that the tetracyclic acetic acid of **10** arises via the polyacetate route, whereas the large side chain is derived from a phenyl pyruvate (via shikimate) unit. This would be in accord with the suggestion of Hendrickson[16] that the condensation of ten acetate units may represent an upper limit for this biosynthetic pathway. The solution to the problem, however, awaits tracer studies.

VI. Summary

A previous structure postulated for thermorubin has been refuted. Instead it has been found to have the novel structure **11**. Thermorubin is a 1*H*-2-anthro[2,3-*c*]pyran derivative, but we feel that, in keeping with what we consider to be its biogenetic origins and for the sake of simplicity in nomenclature, it should be named as an oxanaphthacene,[15] namely, 12-hydroxy-10,11-dimethoxy-3-methoxycarbonyl-8-carboxymethyl-9-[1′-oxo-3′-hydroxy-3′-(2″-hydroxyphenyl)prop-2′-enyl)-1*H*-2-oxanaphthacen-1-one. The numbering of the atoms and the letter designations of the rings follow those currently used for the anthracycline system. The structure of thermorubin suggests that substantial secondary metabolic modifications have occcurred in its biosynthesis.

Chemical Experimental Section[17]

Purification of Thermorubin. A solution of crude thermorubin (1.0 g) in tetrahydrofuran (6 mL) was added to a column (30 × 2 cm) made from a pure chloroform slurry of silica gel (100 g) loaded with 3.4% potassium dihydrogen phosphate. The column was eluted with chloroform (~150 mL) until the tetrahydrofuran was washed out. These washings were discarded, and the column was eluted with 1% methanol in chloroform (200 mL). Evaporation of this solution led to an organic solid which was recrystallized from chloroform to give the chloroform solvate of thermorubin (0.2 g) as rosettes of very small blades. Crystallization of this material from ethyl acetate gave a poor recovery of pure thermorubin as a microcrystalline, orange powder having no definite melting point. The compound turns black at temperatures above 200 °C: UV (EtOH) λ_{max} nm (ϵ) 252 (24 000), 298 (56 760), 325 (53 840), 423 (shoulder, 19 460), 435 (18 490); IR (Nujol mull) 3500–2200, 1730, 1710, 1675, 1610, 1580, 1555, 1490, 1290, 1248, 1200, 1115, 1105, 1080 cm^{-1}; ^{1}H NMR (Me_2SO-d_6, 25 °C) δ 3.95 (2 H, s, ArCH_2CO_2H), 4.04 (6 H, s, OCH_3), 4.12 (3 H, s, OCH_3), 7.11 (3 H, m, ArH and –CH=C), 7.57 (1 H, m, ArH), 8.03 (3 H, d, d, ArH), 8.47 (1 H, s), 8.55 (1 H, s), four broad singlets (OH) exchangeable with D_2O are present at 11.17, 12.64, 13.44, and 16.61; ^{13}C NMR (Me_2SO-d_6, 31 °C, 25.5 MHz) δ 52.69 (–CO_2CH_3), 63.79 (OCH_3), 64.09 (OCH_3), 79.10, 98.89, 103.2, 114.0, 116.1, 117.4, 117.6, 118.8, 119.3, 119.5, 122.8, 126.2, 127.5, 129.2, 129.5, 132.8, 134.4, 135.4, 136.4, 140.1, 153.7, 157.3, 158.7, 159.7, 164.3, 166.0 (–CO_2CH_3), 171.6 (C=O), 183.3 (C=O), 187.1 (C=O); mass spectrum *m/e* (% abundance), 614 (2) $(M + 14)^+$, 600 (3) M^+, 596 (9), 564 (4), 538 (5), 394 (16), 380 (9), 377 (7), 319 (10), 189 (11), 176 (11), 162 (35), 121 (100), 120 (51), 93 (22), 92 (34), 65 (21).

Anal. ($C_{32}H_{24}O_{10}$) C, H.

Trimethylthermorubin. A solution of thermorubin (1.0 g) in tetrahydrofuran was treated within 3 min with a solution of diazomethane (~0.1 mol) in ether (100 mL). The mixture was stirred for 1 h, and the solvents and excess reagent were removed by evaporation. Preparative TLC using five 20 × 40 cm plates (silica gel PF_{254}, CH_2Cl_2–MeOH (99:1 v/v), five cycles) was used to separate the dominant yellow component from four other substances. Elution of the appropriate band by means of 10% MeOH in CH_2Cl_2 yielded trimethylthermorubin (0.47 g), which was recrystallized from methanol to give the pure compound as a microcrystalline, orange powder (0.37 g): mp 121–123 °C; UV (EtOH) λ_{max} nm (ϵ) 253 (27 000), 296 (39 200), 325 (50 800), 403 (6500), 424 (7900); IR (Nujol mull) 3200–2200 (broad), 1740, 1635, 1603, 1492, 1380, 1360, 1308, 1290, 1220, 1165, 1120, 762 cm^{-1}; ^{1}H NMR (C_6D_6, 88 °C, sealed tube) δ 3.48 (3 H, s, OCH_3), 3.59 (3 H, s, OCH_3), 3.60 (3 H, s, OCH_3), 3.76 (2 H, s, ArCH_2CO_2H), 3.80 (3 H, s, OCH_3), 3.84 (3 H, s, OCH_3), 4.05 (3 H, s, OCH_3), 6.01, 7.12, 7.35, 7.59, 7.84 (4 H, 4 s, ArH or –CH=C), 7.79, 7.18, 6.96, 6.68 (4 H, 4 m, ArH), 13.27 (1 H, s, OH); mass spectrum *m/e* (% abundance) 656 (13.7) $(M + 14)^+$, 642 (3.4) (M^+), 610 (93.0), 596 (13.8), 595 (10.0), 578 (47.4), 564 (8.6), 550 (34.5), 522 (17.2), 549 (12.6), 131 (44.8), 121 (100), 105 (22.4).

Anal. ($C_{35}H_{30}O_{12}$) C, H.

Oxidation of Trimethylthermorubin. A solution of trimethylthermorubin (0.1 g) in acetone (30 mL) at 0 °C was treated dropwise over 5 min with a solution of potassium permanganate (0.25 g) in water (2.5 mL). The mixture was allowed to come to room temperature and stirred for 2 h. Precipitated manganese dioxide was removed by filtration, and the filtrate was decolorized by the addition of sodium bisulfite (1 g) and made acidic by the addition of dilute hydrochloric acid. Extraction of the resulting solution with ethyl acetate (3 × 25 mL) followed by drying ($MgSO_4$) and evaporation gave an oily product (0.35 g). Analysis by GC–MS showed this material to be a complex mixture of more than 25 substances, but by far the most dominant (32% of the mixture) material was a compound showing a molecular ion at *m/e* 166. By comparison of the mass spectrum, GC retention time, and NMR spectrum with those of an authentic sample this proved to be methyl 2-methoxybenzoate. Of the other components in the mixture, none was present in sufficient quantity to permit identification.

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Supplementary Material Available: Elemental analyses for designated compounds, atomic coordinates with thermal parameters, and calculated and observed structure factors (10 pages). Ordering information is given on any current masthead page.

(14) This has been reviewed recently: Haslam, E. In "The Shikimate Pathway"; Wiley: New York, 1974.

(15) Richards, J. B.; Hendrickson, J. B. "The Biosynthesis of Terpenes, Steroids and Acetogenins"; W. A. Benjamin: New York, 1964; pp 104–108.

(16) Reference 15, p 95.

(17) NMR spectra were measured on either a Varian HR-100 or a CFT-20, IR spectra were taken on a Perkin-Elmer 257 instrument, and UV spectra were recorded on a Cary 12 spectrometer. Mass spectra were taken on a Hewlett-Packard HP5983 GC/MS spectrometer (low resolution) or a Kratos MS-30 (high resolution). Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.