Thermorubin Biosynthesis: Evidence for the Involvement of both Salicylic Acid and an Undecaketide

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The mixed biosynthesis of thermorubin, a metabolite produced by a thermophilic actinomycete, from salicylic acid (derived from the shikimate pathway) and an undecaketide is demonstrated.

Thermorubin (**1**) is an antibiotic produced by *Thermoactinomyces vulgaris*,[1] a thermophilic saprophytic actinomycete which can synthesize (**1**) from more than 95% of its strains.[2] The structure of thermorubin (**1**), originally thought to be a xanthone–methylene–anthracene,[3] was shown by Johnson *et al.*[4] to be a phenyl–propenyl–oxanaphthacenone.

Thermorubin proved to be very active against gram-positive bacteria, less active against gram-negative bacteria, and virtually inactive against yeasts and filamentous fungi. Also, it is bacteriostatic and inhibits protein synthesis at the level of translation without affecting DNA and RNA syntheses.[5] Several hypotheses have been formulated for its biogenesis.[4]

Results and Discussion

The polyketide origin of 22 of the 29 carbon atom skeleton of thermorubin (**1**) can be demonstrated by a ^{13}C n.m.r. analysis of [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate-derived (**1**) (Scheme). Resonances in the ^{13}C n.m.r. spectrum of thermorubin (**1**) were assigned by chemical shifts and unambiguous pairing of spin–spin coupled carbons in the spectrum of the [1,2-$^{13}C_2$]acetate-derived thermorubin (Table 1).

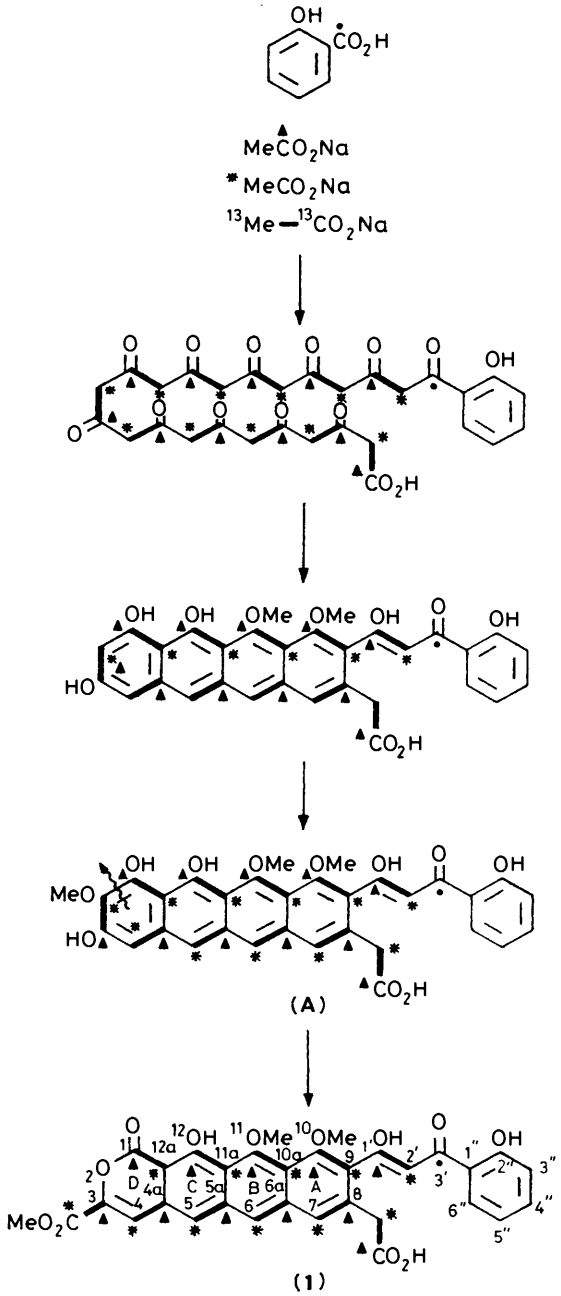
The remaining seven atoms of its skeleton are derived from salicylic acid as shown by incorporation of [1-^{13}C]salicylic acid (Table 1). In THF, thermorubin (**1**) is essentially present in the enol form (shown in the Scheme), which equilibrates very slowly to the corresponding ketone structure. The enol structure was assigned by *X*-ray analysis.[4] The existence of the keto–enol equilibrium can be demonstrated by the appearance of new signals coexisting with the old ones.

The new assignments reported in Table 2 agreed with enrichments and the spin–spin coupling shown by the labelled precursor-derived thermorubin, and confirmed its biosynthetic origin. The most probable sequence of reactions involved in thermorubin biosynthesis is shown in Scheme 1. According to Johnson and co-workers,[4] thermorubin (**1**) is obtained by the degradation of an intermediate molecule such as compound (**A**) in which ring D is cleaved (Woodward oxidative fission) to give a carboxylic acid and a methyl pyruvate residue. Lactonization of these two groups would give ring D of thermorubin (**1**). The possibility that the biosynthesis occurs according to this Scheme has been recently demonstrated chemically.[6]

The mixed biosynthesis either occurs with salicylic acid as the starter unit for the growth of the undecaketide chain or with the condensation between a preformed undecaketide, or its aromatic derivative, and activated salicylic acid.

Experimental

^{13}C N.m.r. spectra were recorded on a XL-200 Varian spectrometer operating at 50.288 MHz for solutions of



Scheme.

Table 1. Assignments of resonance in ^{13}C n.m.r. spectra and isotopic enrichment[a] in thermorubin from cultures containing singly and doubly ^{13}C-labelled acetates and ^{13}C salicylic acid

			Isotopic enrichment (%)			
δ (p.p.m.)	Assignments	$^1J_{C\text{-}C}$ (Hz)	[1-^{13}C]-Acetate	[2-^{13}C]-Acetate	[1,2-$^{13}C_2$]-Acetate	[1-^{13}C]-Salicylic acid
166.93	C-1	74.3	3.4		0.96	
128.20	C-12a	72.0[b]		1.8	0.77[c]	
141.68	C-3	92.9	3.3		0.80	
160.26	*C*-O_2Me	91.9		2.5	0.63[c]	
136.70	C-4a	55.0	2.7		0.79	
113.78	C-4	53.7		2.0	0.72	
137.60	C-5a	58.0	3.3		0.76	
123.71	C-5	60.0		1.8	0.87	
133.70	C-6a	65.1	3.0		0.62	
117.26	C-6	61.8[b]		1.9	0.67[c]	
128.75	C-8	69.0[b]	2.9		0.77[c]	
126.19	C-7	64.2		2.3	0.94	
158.63	C-10	74.3[b]	3.5		0.76[c]	
99.50	C-9	71.5		2.1	0.75	
166.04	C-11	67.3	3.2		0.87	
118.43	C-10a	67.0[b]		0.9	*e*	
155.39	C-12	80.0	3.2		0.77	
119.95	C-11a	78.5[b]		1.7	1.30[c]	
178.40	C-1′	69.0	2.5		0.72	
100.48	C-2′	68.2		2.6	1.01	
171.36	–CO_2H	55.7	3.5		0.13	
38.42	–CH_2CO_2H	55.7		2.3	0.78	
195.12	C-3′					72.9
126.70	1″					
162.85	2″					
135.94	3″					
126.71	4″					
119.07	5″					
119.06	6″					
63.90	OMe					
63.70	OMe					
52.08	CO_2Me					

[a] Enrichments, due to incorporation of labelled precursors, were calculated by means of the formula reported by P. L. Canham, L. C. Vining, A. G. McInnes, J. A. Walter, and J. L. C. Wright, *Can. J. Chem.*, 1977, **55**, 2450. [b] Approximate calculation due to peak overlap. [c] Inexact value due to peak overlap. [d] Values for carbons in parenthesis may be interchanged in couples. [e] Measurement precluded by overlap of multiplets. [f] Values in parenthesis may be interchanged.

Table 2. Distribution of labels in the keto–enol equilibrium

Precursor			
▲MeCO$_2$Na	▲C-1′	178.84 p.p.m.	199.47 p.p.m.
*MeCO$_2$Na	*C-2′	100.48 p.p.m.	54.70 p.p.m.
^{13}Me-^{13}CO$_2$Na	^1J[C(1′)–C(2′)]	68.5 Hz	40 Hz
HOC$_6$H$_4$*CO$_2$H	*C-3′	195.12 p.p.m.	201.2 p.p.m.

thermorubin in [$^2H_8$]THF, acquisition time 0.6 s, time delay 1.5 s, spectral width 12 000 Hz, flip angle 40°, temperature 25 °C. All chemical shifts are quoted as p.p.m. downfield from internal $SiMe_4$.

The distribution of isotopic label in the thermorubin was determined by comparing the ^{13}C n.m.r. spectra of enriched and natural abundance thermorubin recorded under identical conditions.

Culture Conditions.—Submerged cultures of *Th. vulgaris* ATCC 14570 were grown in Erlenmeyer flasks with a medium containing 3% glucose, 1.5% soya meal, 0.25% glycerol, 0.25% sodium glycerophosphate, 0.05% $MgSO_4 \cdot 7H_2O$, and distilled water and incubated at 50 °C on an alternative shaker (100 strokes min^{-1}) for 48 h.

The culture was acidified to pH 3.5 with 1M HCl and thermorubin (**1**) was extracted from the mycelium with $CHCl_3$–THF (9:1) (v/v). The crude extract obtained by standard work-up was washed with hexane, the soluble fraction discarded, and the insoluble residue purified by flash chromatography[7] on silica gel loaded with 3% KH_2PO_4[4] [eluant $CHCl_3$–MeOH (99:1, v/v)]. On concentration of the eluant, thermorubin (**1**) was obtained (*ca.* 100 mg l^{-1} of culture).

Incorporation Experiments.—For labelled precursor feeding experiments sodium [1-^{13}C]acetate (90%, ^{13}C, 750 mg l^{-1}), sodium [2-^{13}C]acetate (92.8% ^{13}C, 380 mg l^{-1}), sodium [1,2-$^{13}C_2$]acetate (93% ^{13}C, 380 mg l^{-1}), and [1-^{13}C]salicylic acid (99% ^{13}C, 200 mg l^{-1}) were added after 12 and 18 h of incubation in two equal portions.

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