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Formation and Characterization of Lumitetracycline-Type Photoproducts from Members of the Tetracycline Family[1]

Gerard Olack and Harry Morrison*

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

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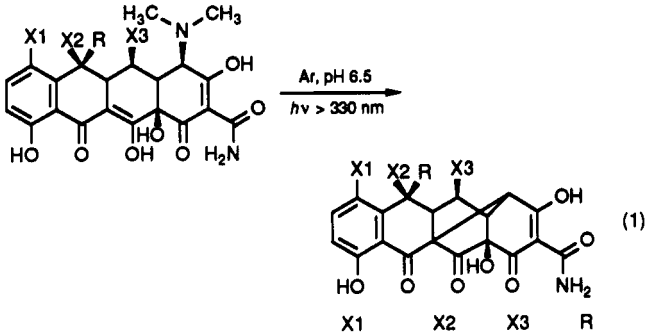
Most of the tetracycline family of antibiotics are known to be phototoxic, but the origin of this effect is not yet understood.[2] For example, it is unclear whether the agent responsible for the dermatoses is the parent tetracycline (Tc) or a photoproduct derived therefrom. It is therefore noteworthy that a Tc photoproduct, lumitetracycline (LTc), has recently been isolated in greater than 70% yield. LTc has been found to form in both organic and aqueous media and to be quenched when the photolysis is conducted in the presence of oxygen.[2] We now report that the Tc to LTc reaction is general for the tetracycline family and has been observed for doxycycline (DOX), oxytetracycline (OTc), chlorotetracycline (CTc), demeclocycline (DEM), and minocycline (MIN) (cf. eq 1).



(1)

		X1	X2	X3	R
tetracycline	(Tc)	H	OH	H	CH_3
oxytetracycline	(OTc)	H	OH	OH	CH_3
doxycycline	(DOX)	H	H	OH	CH_3
chlorotetracycline	(CTc)	Cl	OH	H	CH_3
demeclocycline	(DEM)	Cl	OH	H	H
minocycline	(MIN)	NMe_2	H	H	H

Results and Discussion

Formation and Isolation of Photoproducts. Solutions of CTc, DEM, OTc, DOX, and MIN in pH 6–6.5 phosphate buffer were irradiated under anaerobic conditions with long-wavelength UV light ($\lambda > 330$ nm) to yield photoproducts that were isolated as light yellow powders in yields of 35–49%. HPLC analysis of the photolysate gave evidence for little unreacted starting material, with the exception of MIN where the conversion to LMIN was low (8%) and most of the starting material was found to be present after the photolysis. Both the photolysis and isolation conditions were those developed for the Tc to LTc conversion and thus were not optimized for these other members of the tetracycline family.

Table I. Absorption Maxima for Tetracyclines and Lumitetracyclines[a]

	λ_1	ϵ_1	λ_2	ϵ_2	λ_3	ϵ_3
Tc	363	13800	268	18600		
OTc	357	12600	267	20900		
DOX	351	13200	267	17400		
CTc	355	10000	270	10000		
DEM[b]	370	12309	267	18356		
MIN[b]	357	13361	265	18040		
LTc	340	4440	265	20800	212	17400
LOTc	337	4840	266	23000	209[c]	20000
LDOX	343	4930	269	25800	209[c]	20100
LCTc	355	4780	265	21100	224	20000
LDEM	353	5010	264	23400	220	21900
LMIN[d]	378	5300	269	38000		

[a] All data for 0.01 N HCl in methanol unless otherwise noted; tetracycline values from ref 3. [b] Determined in these laboratories by Changhe Xiao. [c] Base-line drift in the 200–210 region may have affected this value. [d] Determined in MeOH.

Spectroscopy. UV absorption spectra of the lumi compounds were taken in 0.01 N HCl in methanol, and the results are presented in Table I, together with UV data for several of the tetracyclines drawn from the literature.[3] The UV data for the photoproducts are generally consistent throughout the series, with a longer wavelength band at 330–350 nm having an extinction coefficient of ca. 5000, and a second maximum at 265–270 nm with an extinction coefficient of ca. 20 000. All the lumi products show a marked reduction in the extinction coefficient associated with λ_1, the transition centered in rings BCD (the aryl ring is ring D). LOTc and LDOX also show a blue shift in λ_1 relative to their parent substrates, as is seen for LTc relative to Tc. Both effects are anticipated as a consequence of the change in the BCD chromophore upon forming the C11a to C4 bridge.

In every case the mass spectrum of the lumi product contains a molecular ion 45 amu less than the mass of the parent compound, consistent with the net loss of dimethylamine in each of the transformations. The ^1H NMR spectral data for the lumi products in acetone-d_6 are presented in Table II and in each case one observes (1) the number of aliphatic and aromatic protons anticipated for a parent compound minus dimethylamine, (2) the presence of the hydroxyl, phenol, amide, and 3-enol protons, but the absence of a resonance corresponding to the 12-enol hydrogen (expected at δ 13–15), and (3) downfield shifts associated with the bridgehead H4 and H4a resonances. The assignments of the aliphatic region resonances were based on matching their coupling patterns to the coupling patterns for the parent tetracyclines (there is an excellent correlation among the coupling constants for the various lumi derivatives). The aromatic region resonances were assigned by comparison of the chemical shifts to those observed for Tc.[2]

Proton-decoupled ^{13}C NMR and APT (attached proton test) spectra were obtained for each of the photoproducts in acetone-d_6, with the exception of LMIN for which insufficient material was available. The ^{13}C chemical shifts are summarized in Table III. Calculated chemical shifts derived from the LTc spectral data, with adjustments for

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Table II. Summary of the ^1H NMR Assignments for Lumitetracycline Derivatives[a]

proton	LTc	LOTc	LDOX	LCTc	LDEM	LMIN
6-Me	1.71	1.84	1.57	2.03		
6α					5.35	2.84
6β			3.27			3.68
5-H_2	2.42				2.47	
α	2.33[b]	4.93	4.48	2.47		1.87
β	2.38[b]			2.48		2.55
5a	2.66	2.44	2.26	2.73	2.87	2.84
4a	3.22	3.39	3.41	3.25	3.27	3.23
4	4.19	4.08	3.7	4.11	4.25	3.85
OH[c]	4.89	4.31/4.34	4.94	5.37	5.45	
OH[c]	5.37	5.02	6.2	5.43		
OH[c]		6.12				
9	6.95	6.94	6.87	7.00	7.02	6.80
7	7.19	7.17	7.04			
8	7.62	7.61	7.62	7.64	7.68	7.64
amide	8.16	8.21	8.27	8.16	8.15	8.2
	8.93	8.99	9.15	8.97	8.99	9.1
phenol	12.23	12.03	12.19	12.85	12.11	11.9
3-enol	18.27	18.0	17.3	18.0	18.0	17.8

[a] All data in acetone-d_6; LTc data from ref 2. [b] 5α and 5β distinguishable in CD_3OD. [c] No distinction can be made between 5-OH, 6-OH, and 12-OH.

Table III. Summary of the ^{13}C NMR Assignments for Lumitetracycline Derivatives[a]

carbon	LTc	LOTc	LDOX	LCTc	LDEM
5	24.1	72.9	77.5	24.7	24.3
6-Me	24.6	23.9	14.6	28.1	
5a	49.1	54.5	50.0	49.5	44.7
4a	52.8	58.1	57.1	52.3	52.8
4	54.0	52.3	54.3	54.4	55.1
11a	66.4	65.1	65.7	65.8	65.5
6	70.9	70.4	34.4	73.0	65.8
12a	88.3	88.8	88.4	88.4	88.5
2	98.8	98.2	97.0	98.8	98.7
7	116.7	116.2	116.4	123.3	124.0
10a	116.7	116.6	117.3	118.4	117.9
9	118.4	118.3	117.2	120.5	120.4
8	138.8	138.6	138.7	142.5	139.6
6a	149.2	148.9	147.6	144.5	142.5
10	163.7	163.4	163.6	163.6	162.6
amide	173.5	173.5	173.4	173.7	173.7
1,3,11	187.3	186.3	185.6	187	187.3
1,3,11	197.3	196.3	195.3	198	196.1
1,3,11	199.6	199.1	197.9	200.1	199.7
12	204.9	203.7	204.9	204.9	204.4

[a] In acetone-d_6.

the presence or absence of substituents,[4] show excellent agreement with observed chemical shifts in all cases. In particular, the chemical shifts for the key positions in the newly generated norbornyl ring system of the lumi structures (i.e., the bridgeheads at C4a and C11a, and the bridging carbon, C4), which change significantly upon conversion of the Tc family to these photoproducts, are nearly identical with those observed in the parent LTc for all of the new lumi products studied.

Quantum Efficiencies. The quantum efficiencies for the formation of lumi products and for the loss of the parent tetracycline were determined at pH 6.5 (the optimal pH for lumi product formation), in deoxygenated solutions, with 366-nm light. The results are presented in Table IV. The relative efficiencies for formation of the lumi products is Tc > DOX > OTc > CTc > DEM. The disappearance of starting material generally mirrors this trend, except for the inversion of the CTc/DEM sequence. The mass balance is good for Tc, DOX, and OTc (78, 88, and 72%, respectively). This is not the case for CTc and DEM (37 and 21%, respectively); for these drugs, most of the reacted starting material apparently results in the formation of other major photoproducts, possibly "anhydro" derivatives.[5] When broad-band (e.g., a uranium yellow filtered mercury arc; λ > 330 nm) light was used, with the tetracyclines in degassed phosphate buffer at physiological pH (7.4), the relative rates for lumi product formation were found to be DOX > OTc > Tc > CTc > DEM > MIN.

Table IV. Quantum Efficiencies for Formation of Lumi Products and for Tetracycline Destruction[a]

compd	φ loss ($\times 10^{-3}$)	φ lumi ($\times 10^{-3}$)
Tc[b]	7.3	5.6
DOX	2.0	1.8
OTc	1.9	1.4
CTc	0.86	0.31
DEM	1.4	0.30

[a] Photolyses in degassed aqueous solutions, pH 6.5, using 366-nm light. [b] Photolysis at pH 6.0.

Conclusions

The formation of lumitetracycline structures is general to the tetracycline family. The role, if any, of these compounds in the phototoxicity phenomenon is yet to be determined. However, there is evidence that LDOX can be phototoxic, at levels comparable to the parent DOX, as evidenced by mitochondrial fragmentation and the alteration of mitochondrial membrane potential upon photolysis with UVB light.[6] In contrast, the treatment of mice with LDOX followed by the application of a quantitative mouse-tail technique, which measures photoinduced edema, gave no evidence for phototoxicity.[7] Mechanistic studies on the conversion of tetracyclines to lumitetracyclines, and related photophysical studies of the parent substrates, are in progress.

Experimental Section

The experimental procedures and detailed spectral data are presented in the Doctoral Dissertation of G.O.

Preparation of the Lumi Photoproducts. A solution of the parent tetracycline salt (120–130 mg, 100 mg for MIN·HCl) in 0.1 M phosphate buffer (ca. 125 mL), pH 6–7, was deoxygenated

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and irradiated in the reaction vessel for 10–13 h (2 days for MIN), at room temperature (23 °C) with continuous argon degassing. The photolysate was acidified with 10 mL of 4 N HCl, 10 mL of brine was added, and the solution was extracted with EtOAc (4 × 20 mL). The combined extracts were washed with 25 mL of H_2O, dried (anhyd Na_2SO_4), filtered through glass wool, and evaporated to dryness under vacuum. The crude lumi product mixtures were purified using an E. M. Merck Lichrosorb RP-8 (size B, 43–60 μM) LoBar column. Eluent conditions were 10% v/v MeCN, 30% MeOH, and 60% 0.2 M NH_4OAc (pH 7), flow rate 6 mL/min, monitored at 272 nm. The mixtures were dissolved in 2–3 mL of MeOH and diluted to twice the volume with the buffer component of the eluent used. The resultant solution was loaded on to the column in 1–1.5-mL increments, injected over a period of up to 5 min. The lumi product was the first major peak to elute from the column, with the onset at 70–100 mL (solvent front ca. 50 mL) and the product completely eluted at ≤200–300 mL. The fraction containing the lumi product was acidified with up to 10 mL of 4 N HCl, concentrated in vacuo to 40–50% of the original volume, and extracted 4 times with 15–20-mL fractions of EtOAc. The combined fractions were washed once with 20 mL of H_2O, dried, treated with carbon black, and filtered through a medium-fritted glass funnel. The solution was evaporated in vacuo to ca. 3–4 mL, and ca. 150–300 mL of hexanes was added to precipitate the lumi products as flocculent off-white to yellow precipitates. These were collected through a 13-mm Nylon 66 0.45 μm membrane (Alltech) and dried to give isolated amounts of the lumi products ranged from 40 to 60 mg (ca. 8 mg for LMIN).

Quantum Efficiency Determinations. This experiment utilized the 366-nm mercury line isolated with a soft glass sleeve surrounding the lamp and a Corning CS7-37 366-nm bandpass filter. Quartz photolysis tubes (7.5 cm i.d.) were placed in a Merry-go-round (New England Ultraviolet Co.) mounted in front of the bandpass filter. Photolyses were carried out for 3 h at room temperature (22–25 °C) with benzophenone/benzhydrol actinometry.[8] Stock solutions of 0.5 mM tetracycline (OTc, DOX, CTc, DEM) in 50 mM phosphate buffer, pH 6.4, ionic strength of 0.15 (adjusted with NaCl), were prepared which had an absorbance of ≈3 at 366 nm (1-cm path length). Aliquots (5 mL) from each solution were placed in test tubes and covered with aluminum foil to serve as dark controls. Two 5-mL aliquots of each of the tetracyclines and of the actinometer solution were placed into the tubes and deoxygenated with Ar for 15 min, and the tubes were sealed with rubber septa. Analyses were by reverse-phase HPLC with an Alltech Econosil C-18 (4.6 mm × 25 cm) 10 μm column, using propiophenone as an internal standard.

Acknowledgment. We thank the National Institutes of Health (Grant 5 RO1 AR39286) for support of this research.

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Cytotoxic Metabolites of the Marine Sponge *Mycale adhaerens* Lambe[1]

Nobuhiro Fusetani,* Takeo Sugawara, and Shigeki Matsunaga

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Hiroshi Hirota

Department of Chemistry, Faculty of Science, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

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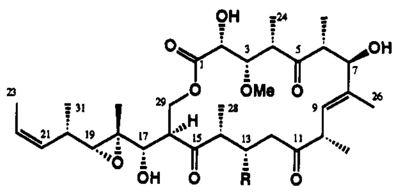
Marine sponges of the genus *Mycale* are a source of novel bioactive nitrogenous metabolites; e.g., mycalysins A and B,[2] mycalamides A and B,[3] and mycalolides A–C.[4] In our continuing search for cytotoxic metabolites from Japanese marine invertebrates, we found that the lipophilic extract of the marine sponge *Mycale adhaerens* showed potent cytotoxic activity. Bioassay-guided isolation yielded five active compounds, of which 13-deoxytedanolide and a brominated isocoumarin named hiburipyranone are new. This paper deals with the isolation and structure elucidation of these compounds.

The EtOH extract of the sponge was partitioned between water and ether. The ether phase was fractionated by flash chromatography on silica gel, followed by gel filtration and reversed-phase HPLC to afford 13-deoxytedanolide (**1**, (5.5×10^{-3})% based on wet weight) and hiburipyranone (**3**, (2.1×10^{-4})%), together with three known compounds, mycalolides A (**4**, (2.1×10^{-4})%) and B (**5**, (6.8×10^{-5})%)[4] and a bromoindole **6** ((3.2×10^{-5})%).[5] The known compounds were identified by comparing spectral data with those reported in literature.



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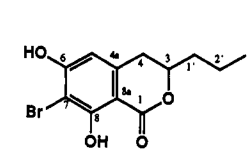

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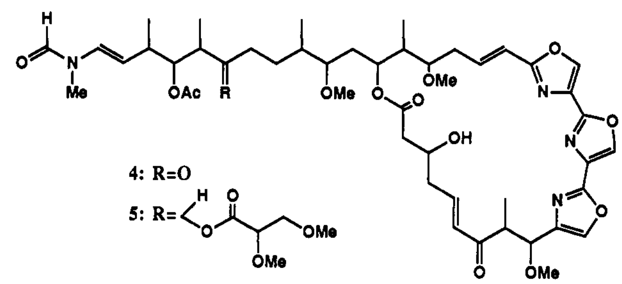

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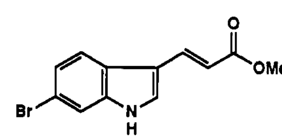

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The new compound **1** had a molecular formula of $C_{32}H_{50}O_{10}$, which was established by the high-resolution FAB mass spectrum. The ^{1}H and ^{13}C NMR spectra indicated that **1** might be a macrolide containing three ketones that interrupted ^{1}H NMR coupling sequences. However, the ^{1}H NMR spectrum showed well-resolved signals and interpretation of the COSY and C–H correlation[6] spectra gave rise to four partial structures, a–d, which were eventually connected through ketones by tracing cross-peaks in the HMBC spectrum.[7]

The assignment of the C2–C4(C24) portion (partial structure a) was straightforward by interpretation of the

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