Lumitetracycline: A Novel New Tetracycline Photoproduct[1]

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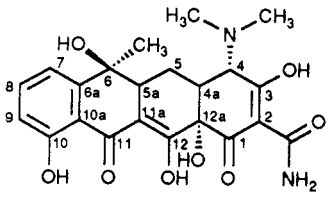
A new photoproduct ("lumitetracycline", LTc), formed by the irradiation of the phototoxic antibiotic tetracycline (Tc) in organic or aqueous media, has been isolated in good yield. Its structure has been deduced from spectroscopic data, primarily one- and two-dimensional NMR techniques. The key structural feature of this photoproduct is the formation of a new carbon–carbon bond between C-4 and C-11a, thus generating a norbornyl ring system within the molecule.

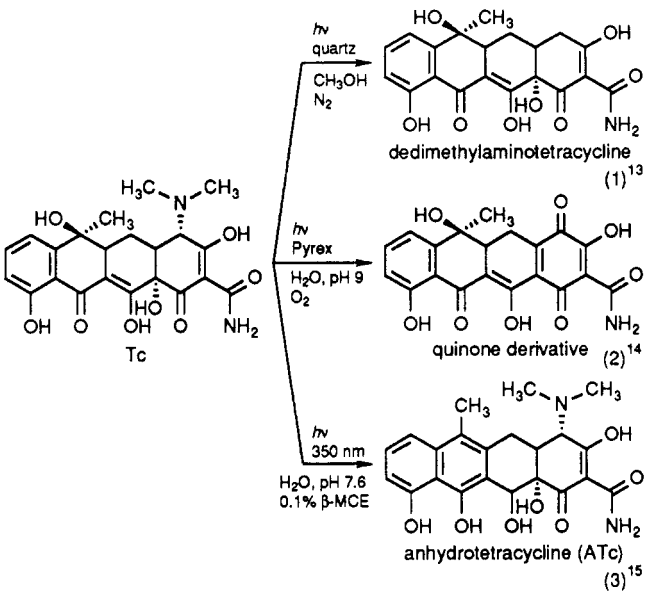
Drug-induced phototoxic dermatoses are characterized by a morphology which often resembles an exaggerated sunburn, occur on the first combined drug and light exposure, and are elicited in all people receiving adequate doses of the drug and appropriate wavelengths of radiation.[2] There are large numbers of chemicals that have been identified as phototoxic, including antibiotics, contraceptives, tranquilizers, and artificial sweeteners, but a detailed understanding of the origin of their phototoxicity at the molecular level is unavailable for all but a very few of these substrates. Tetracycline (Tc) and its analogues,



tetracycline (Tc)

one of the most widely prescribed family of antibiotics, represent a virtually classic example of such phototoxicity.[3,4] Not all of the family are equally potent; 6-demethyl-7-chlortetracycline is the most phototoxic (virtually everyone taking the drug is sensitized to light![5]) while 6-demethyl-6-deoxy-7-(dimethylamino)tetracycline is essentially free of such side effects.[6] Despite the fact that Tc phototoxicity has been known for many years, the primary photochemical events which bring about the phototoxic response are still unknown. The culpable agent may be Tc itself or a photoproduct,[7] and possibilities for the initial biological target include lipids, proteins, and DNA.[8] Cell membranes have been implicated,[7] but more recent results suggest mitochondrial damage as the initial event.[9] Tritiated tetracycline can be photolytically covalently bound to eukaryotic and prokaryotic ribosomes,[10,11] with both protein and RNA found to have taken up the label.[10] There is a report that co-photolysis of Tc and DNA creates new lesions, apparently involving guanine residues, and causes backbone cleavage which is attributed to hydroxy radicals.[12] There is appreciable evidence that singlet oxygen can play a significant role in Tc-induced damage.[4,7,9,12]

As noted above, one or more photoproducts of Tc may well be a source of the phototoxic response, and it is surprising how little is currently known about Tc photochemistry. Those photoproducts which have been reported are shown in eqs 1–3. We now report the isolation and identification of a new and rather novel tetracycline photoproduct which we have termed "lumitetracycline"



(LTc). There is evidence that this product is itself appreciably phototoxic.[16] We have also isolated photopro-

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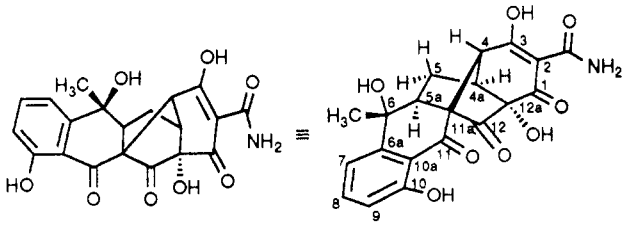
ducts from chlorotetracycline, oxytetracycline, doxytetracycline, and demethylchlorotetracycline, all having NMR spectra consistent with their assignment as LTc analogues.



lumitetracycline (LTc)

Results and Discussion

Formation and Isolation of LTc. The tetracyclines typically contain two distinct chromophores, the β-tricarbonyl system in ring A absorbing at 262 nm and the aryl β-diketone chromophore (rings B, C, D) absorbing at 225, 285, 320, and 360 nm (the latter being the source of the drug's yellow color).[17] In the initial study, the photolysis of Tc was carried out in organic media (acetonitrile) using long-wavelength ultraviolet light (uranium yellow filter; $\lambda > 330$ nm) in a continuously argon degassed medium at ca. 10 °C. The formation of a new product was evident by HPLC, and LTc could be recovered as a yellow amorphous powder in isolated yields ranging from 30 to 40% after low-pressure reverse-phase chromatographic workup (yields approaching 50% are obtainable with added acetic acid as a catalyst). LTc is also formed in acetone and methanol, albeit less cleanly. Photolysis in aqueous solution buffered with phosphate at pH 6.4, initially degassed with argon and with the light source described above, also gives rise to LTc at an even greater rate than that observed in acetonitrile and in yields of ca. 70% (cf. eq 4).

$$\mathrm{Tc} \xrightarrow[\substack{\lambda > 330\ \mathrm{nm}\\ \mathrm{argon}\\ \mathrm{CH_3CN\ or\ H_2O(pH\ 6.4)}\\ 10\ ^\circ\mathrm{C}}]{h\nu} \mathrm{LTc} + \mathrm{Me_2NH} \qquad (4)$$

It is noteworthy that neither of the photoproducts indicated in eqs 1 and 2 are formed in significant amounts under the conditions given in eq 4. Dedimethylaminotetracycline formation (eq 1) requires high-frequency light, while quinone formation (eq 2) is only reported for oxygenated solutions. Appreciable amounts of anhydrotetracycline (ATc) are only observed when β-mercaptoethanol is present (cf. eq 3), and this compound is a minor component of the product mixture at pH 6.4 (ca. 5% of LTc). Because LTc production drops rapidly beyond pH 7.0, while the ATc reaction is less pH dependent, the LTc/ATc ratio diminishes in more basic media and is ca.

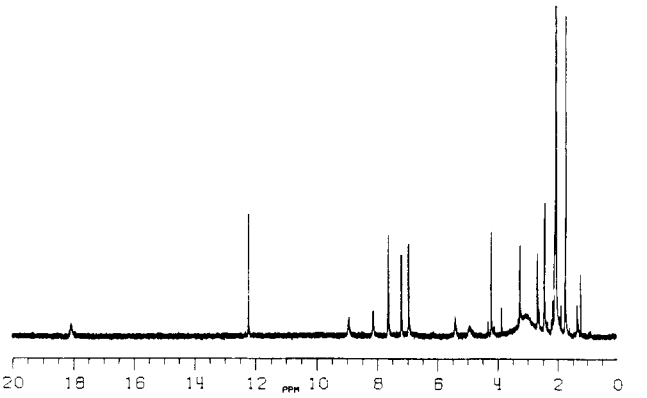


Figure 1. Proton NMR spectrum of LTc in acetone-d_6.

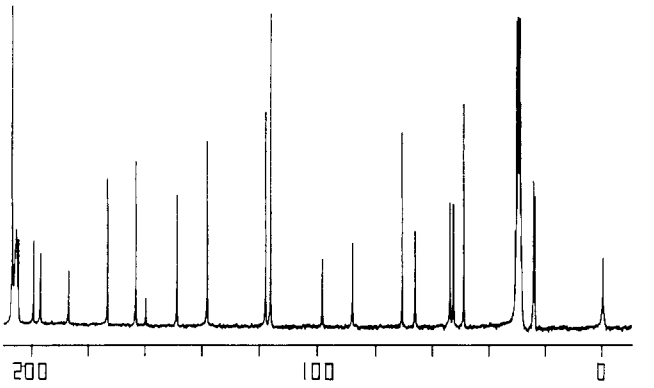


Figure 2. ^{13}C NMR spectrum of LTc in acetone-d_6.

unity at pH 7.6. Dimethylamine has been trapped as the *N,N*-dimethylbenzamide by addition of benzoyl chloride to an acetonitrile photolysis solution in quantities which correlate well with the quantity of LTc formed. There is no evidence for the formation of LTc under any of the above sets of conditions in the absence of light.

Mass Spectrometry. Both positive chemical ionization (+CI) and fast atom bombardment (FAB) mass spectrometry give evidence for a molecular weight of 399 amu. This corresponds to the net loss of dimethylamine from Tc, a result confirmed by an exact mass determination for the CI derived M + H^+ ion of 400.10496 amu (theory = 400.10497 amu for $C_{20}H_{17}NO_8$).

Ultraviolet Absorption Spectroscopy. A comparison of the absorption spectra of Tc and LTc indicates that the principal change is a blue shift in the long-wavelength band associated with the rings B, C, D chromophore, i.e., in 0.1 M HCl/methanol, the Tc absorption maximum at 362 nm ($\log \epsilon = 4.13$) is replaced in LTc by a maximum at 344 nm ($\log \epsilon = 3.71$) (both retain the maximum at 270 nm). Several Tc derivatives show similar changes, in each case as a consequence of the strain on rings B and C, or bonding at C_{11a}, forcing the β-dicarbonyl in rings B and C to shift from the planar enol form to a "shortened" dicarbonyl chromophore. The comparative data are presented in Table I.

Nuclear Magnetic Resonance Spectroscopy. First-Order Analysis. Proton and carbon NMR spectra of LTc were obtained in several solvents with the results in acetone-d_6 presented in Figures 1 and 2 (see the structures given earlier for numbering). The carbons in the aliphatic region were assigned using heteronuclear correlation experiments (Figure 3); the proton couplings were ascertained using homonuclear correlation experiments (Figure 4), decoupling experiments, and a computer simulation of the 1D spectrum in acetone.

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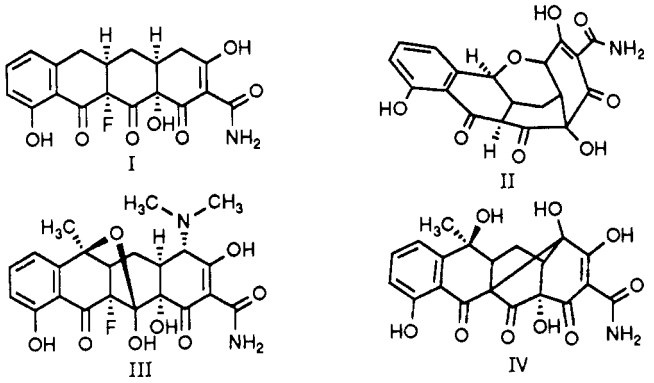
Table I. Absorption Maxima of Some Bridged Derivatives of Tc



compd	λ_{max}	log ϵ	λ_{max}	log ϵ
Tc[a]	270	4.23	362	4.13
LTc[a]	270	4.36	344	3.71
I[b,c]	265	4.40	348	3.57
II[b,d]	258	4.34	338	3.67
III[b,e]	265	4.45	336	3.71
IV[b,f]	268	4.42	346	3.66

[a] In 0.1 M HCl–methanol. [b] In 0.01 M HCl–methanol. [c] Reference 18. [d] Reference 19. [e] Reference 20. [f] Reference 21.

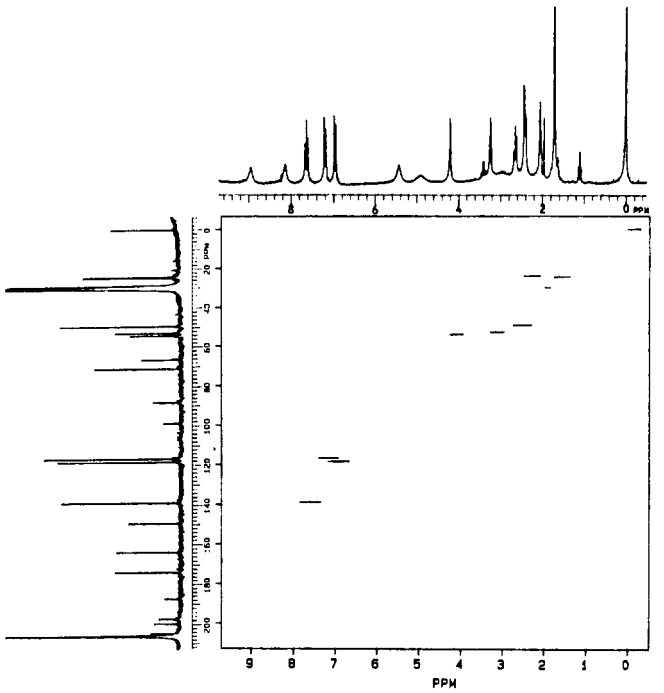


Figure 3. HETCOR spectrum of LTc in acetone-d_6.

(i) Carbon NMR. The ^{13}C chemical shifts for LTc are presented in Table II and may be compared with those for Tc in Table III.[23] The carbon assignments for LTc were correlated with Tc using their relative chemical shifts and the number of attached protons (via the attached proton test, heteronuclear correlation, and a fully coupled spectrum).

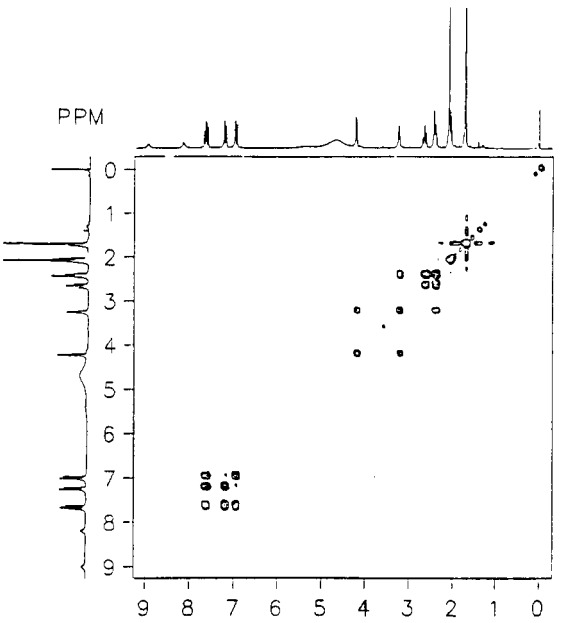


Figure 4. HOMCOR spectrum of LTc in acetone-d_6.

Table II. ^{13}C NMR Spectral Data for LTc

position	m	J(C–H), Hz	δ^a	δ^b
5	t	136.8	24.1	23.0
6-methyl	q	127.1	24.6	23.3
5a	d	133.4	49.1	47.8
4a	d	150.3	52.8	51.6
4	d	152.2	54.0	52.9
11a (12a)	s		66.4	65.3
6	s		70.9	70.0
12a (11a)	s		88.3	87.2
2	s		98.8	97.7
10a	s		116.7	
7	d	170.1	116.7	
9	d	164.4	118.4	
8	d	160.8	138.8	137.9
6a	s		149.2	147.8
10	s		163.7	162.5
amide	s		173.5	172.2
3, 1, 11	s		187.3	186.0
	s		197.3	196.4
	s		199.6	198.6
12	s		204.9[c]	204.2

[a] In acetone-d_6. [b] In acetonitrile-d_3. [c] This peak is partially obscured by acetone but is clearly observed in acetonitrile.

The 20 peaks identified in the spectrum of LTc confirm the carbon count in the molecular formula obtained from the mass spectrum. The five sp^2 C–O peaks seen in Tc are also present in LTc. The amide carbon is assigned to the δ 173.5 peak since it is the most upfield of the group (as it is in Tc) and is within 1 ppm of the Tc signal. The other four peaks require more information in order to be specifically assigned (see below), but the C-12 peak in Tc at δ 176.7 is, in any event, shifted significantly downfield in LTc, indicating a significant chemical change at this position. The chemical shifts and multiplicities of the aromatic peaks of LTc are quite similar to those in Tc and are assigned on the basis of this similarity. Although carbons 10a and 9 of LTc cannot be differentiated in the decoupled ^{13}C spectrum, they can be differentiated by the coupled and APT spectra. The δ 24.1, 24.6, 70.9, and 98.8 signals are assigned to the C-5 methylene, C-6 methyl, C-6, and C-2 carbons, respectively, by analogy with Tc and the expected number of attached protons. These assignments are supported by the correlation experiments discussed below. Of the five remaining peaks, three have hydrogens (δ 99.1, 52.8, and 54.0) and two do not (δ 66.4 and 88.3);

(23) We have reversed the literature[22] assignments for C-9 and C-7 to make them consistent with heteronuclear correlation and coherence transfer experiments on Tc.

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Table III. ^{13}C NMR Spectral Data for Tc[a]

position	δ^b	δ^c
5	22.36	22.9
6-methyl	22.83	23.3
4a	37.06	38.8
5a	40.00[d]	42.0
$N(CH_3)_2$	42.35	43.3
6	68.05	69.3
4	69.58	70.9
12a	74.23	75.1
2	98.29	98.9
11a	105.74	106.5
10a	114.30	115.9
7	115.34	116.1
9	116.81	117.7
8	136.35	137.1
6a	147.97	148.9
10	161.33	162.9
$CONH_2$	172.54	174.5
12	176.70	176.9
1,3	192.26	193.6
		194.2
11	192.84	194.8

[a] Shifts and assignments were confirmed by comparison with the literature.[22] [b] In DMSO-d_6. [c] In acetone-d_6 (acetone reference, 29.8 δ). [d] The peak was obscured by DMSO; the literature value is given.

Table IV. ^1H NMR Spectral Data for LTc

position	m	acetone-d_6[a]	CD_3OD[b]
6-methyl	s	1.71	1.60
5-H_2 (α)	mt	2.42	2.33
5-H_2 (β)			2.38
5a-H	qt	2.66	2.56
4a-H	mt	3.22	3.10
4-H	d	4.19	4.13
6- or 12a-OH	br s	4.89	
		5.37	
9-H	d	6.95	6.95
7-H	d	7.19	7.16
8-H	t	7.62	7.60
amide[c] (a)	br s	8.16	
amide[c] (b)	br s	8.93	
10-phenol	s	12.23	
3-enol	br s	18.27	

[a] Acetone reference at δ 2.04. [b] Methanol reference at δ 3.30. [c] The intramolecularly hydrogen bonded amide hydrogen is designated "b"; the non-hydrogen bonded proton is "a".

their assignments are discussed in conjunction with the proton NMR. However, it is apparent that C-11a and C-4 have changed chemically. The δ 105.74 peak (C-11a) seen in Tc is absent in the LTc spectrum, and, since all the downfield carbons have already been accounted for, the C-11a resonance has to have been shifted upfield. The δ 69.58 signal in Tc is assigned to the methine carbon, C-4, but no methine is found in that region in the LTc spectrum. However, an extra methine is seen at higher field. Both upfield shifts are accommodated in LTc by a deamination of C-4 and the transformation of C-11a from an sp^2 center in Tc to an sp^3 center in LTc.

The C–H coupling constants and multiplicities reported in Table II were derived from a fully coupled ^{13}C spectrum. Most of the values obtained are unexceptional, but two of the aliphatic carbons are associated with coupling constants of 152.2 and 150.3 Hz. These large coupling constants are a strong indication that significant strain exists at these positions.

In summary, both Tc and LTc contain one methyl, one methylene, and six methine (three aromatic and three aliphatic) carbons, if the amino group of Tc is ignored. Assuming LTc has the formula $C_{20}H_{17}NO_8$, there must be one less exchangeable hydrogen in the photoproduct relative to Tc. This requires that one of the hydroxyl groups is deprotonated in the photoreaction.

Table V. ^1H NMR Spectral Data for Tc[a]

position	m	DMSO-d_6	acetone-d_6[b]	CD_3OD[c]
6-methyl	s	1.5	1.6	1.60
5-H_2 (α)	mt[d]	2.0	2.2	1.94
5-H_2 (β)				2.10
$N(CH_3)_2$	br s	2.4	2.5	2.68
4a-H	mt	2.6	2.6	2.63
5a-H	qt	2.8	3.1	2.95
4-H	br d	3.2	3.3	3.43
6- or 12a-OH	br s	4.9	4.2	
9	d	6.9	6.8	6.9
7	d	7.1	7.1	7.1
8-H	t	7.5	7.6	7.5
amide[e] (a)	br s	8.6	8.0	
amide[e] (b)	br s	9.0	9.1	
10-phenol	s	11.7	12.1	
12-enol	br s	13.6	15.2	
3-enol	br s	18.5	18.5	

[a] The assignments are based on the literature values for tetracycline in DMSO-d_6.[24-26] [b] Acetone reference, δ 2.04. [c] Methanol reference, δ 3.30. [d] A pair of quartets in methanol-d_4. [e] (a) Non-Hydrogen bonded, (b) hydrogen bonded.

Table VI. Interpretation of the Homonuclear Correlation Spectrum of Ltc[a]

peak (δ)	position	coupled to δ	connectivity
1.71	6-methyl	none	
2.42	5-H_2	2.66, 3.22	5-H_2 → 5a-H; 5-H_2 → 4a-H
2.66	5a-H	2.42	5a-H → 5-H_2
3.22	4a-H	2.42, 4.19	4a-H → 5H; 4a-H → 4H
4.19	4-H	3.22	4-H → 4a-H
6.95	9-H	7.62	9-H → 8-H
7.19	7-H	7.62	7-H → 8-H
7.62	8-H	6.95, 7.19	8-H → 9-H; 8-H → 7-H

[a] In acetone-d_6, cf. Figure 4.

(ii) Proton NMR. The proton NMR spectrum of LTc confirms many of the above conclusions. The data are presented in Table IV, and the corresponding data for Tc are presented in Table V for comparison. The phenolic and 3-enol protons are clearly present in LTc as are the aromatic hydrogens (the latter have the same coupling pattern and almost the same coupling constants and chemical shifts as those found in Tc). There is also evidence for the 6-methyl, the 5-H_2 methylene and the three methine (4, 4a, 5a) protons in the aliphatic region seen in Tc. Particularly noteworthy is the absence of the 12-enol and the six dimethylamino protons, thus confiriming that the primary change in structure upon formation of LTc is occurring at positions 4 and 12.[27]

The proton coupling patterns for LTc may be extracted from the Homonuclear Correlation Spectrum (Figure 4) and are collated in Table VI. Of principle interest is the coupling pattern: CH(δ 4.19)–CH(δ 3.22)–CH_2(δ 2.42)–CH(δ 2.66), analogous to the 1–1–2–1 pattern observed in Tc. This supports the assignment of these peaks to positions 4, 4a, 5, and 5a in LTc, respectively. The proton coupling constants in LTc and Tc were extracted from the 1D proton and decoupled spectra and are presented in Table VII. These data are from methanol-d_4 wherein the 5-methylene hydrogens in LTc are resolved by ca. 20 Hz.

The coupling patterns in LTc at δ 3.10 and 2.35 are particularly complex, and the chemical shifts and coupling constants for the 4a, 5a, and 5 hydrogens were obtained by simulating the δ 2.3–2.6 region using an iterative pro-

(27) The methine proton at δ 4.2 assigned to the 4-H in LTc is unusually deshielded, possibly a consequence of the close proximity of the hydroxyl group at C-6 (2–3 Å) and the carbonyl group at C-3.

Table VII. Proton Coupling Constants (J) for Tc and LTc[a]

(x,y)	Tc, Hz	LTc, Hz
(8,9)	7.6	7.59[b]
(7,8)	8.4	8.36[b]
(5α,5β)[d]	−13.0[e]	−13.06[e]
(5α,5a)	10.6	5.57[c]
(5α,4a)	12.5	4.33[c]
(5β,5a)	5.8	8.79[c]
(5β,4a)	3.1	0.62[c]
(4,4a)	2.4	2.26[b]

[a] In methanol-d_6. [b] Coupling constants from single frequency decoupled spectra. [c] Coupling constants determined by computer simulation. [d] The 5α-H is upfield of 5-β and is presumed to be anti to 5a and 4a in both Tc and LTc, based on the expected coupling constants of the proposed structures. [e] These values are assumed to be negative, as is characteristic of J_{gem}.

Table VIII. Heteronuclear Correlation[a,b]

H, δ	coupled to	C, δ	assignment
1.71		24.6	Me
2.42		24.1	5
2.66		49.1	5a
3.22		52.8	4a
4.19		54.0	4
6.95		118.4	9
7.19		116.7	7
7.62		138.8	8

[a] In acetone-d_6, cf. Figure 3. [b] There is an additional correlation at δ2(H)/δ30(C) caused by the acetone, and resonances due to traces ethyl acetate are present in the proton spectrum.

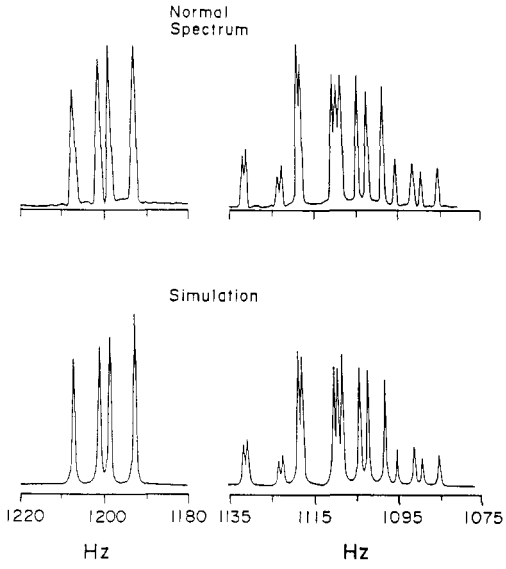

Figure 5. Computer simulation of the LTc ^{1}H NMR spectrum in methanol-d_4.

gram based on the Fortran program LAME.[28] When compared to the experimental spectrum (Figure 5) the average difference in chemical shifts of each line is 0.002 Hz with a standard deviation of 0.054 Hz.

The heteronuclear correlation spectrum (Figure 3), which positively correlates the carbons with their attached protons, provides the assignments given in Table VIII. Note that it is the carbons with the high C–H couplings of 152.2 and 150.3 Hz which correlate to the proton peaks at δ 4.19 (H_4) and 3.22 (H_{4a}), respectively, consistent with the assignments in Table II.

Nuclear Magnetic Resonance Spectroscopy. Confirmation of C–C Connectivity. (i) "Insensitive Nuclei Assigned by Polarization Transfer (INAPT)" Spectra.[29,30] This methodology allows one to utilize two- and three-bond C–H couplings to establish C–C connectivity. Four-bond interactions are rare and only seen when in unsaturated systems.[29,31] The data are shown in Table IX. The data in Table IX provide excellent support for the assigned structure. Most importantly, the new bond between C-4 and C-11a is demonstrated by polarization transfers involving H_{4a}/C-11a, H_4/C-11a, H_4/C-12, H_4/C-5a, and H_{5a}/C-4, none of which would be observable without this new bond. (Note that, as expected, the only four-bond polarization transfers observed involve the aromatic system). Polarization transfer from H_4 to C-11 is the only anticipated transfer not seen. This study also provided the evidence needed to assign the C-12 signal (cf. Table II). Polarization transfers from H_{5a}, H_{4a}, and H_4 all enhance the carbonyl carbon signal at 204.8 ppm, and the only carbonyl carbon within three bonds of each of these protons is C-12.

Table IX. Polarization Transfers from INAPT Experiments[a]

position	H, δ	C, δ	position	no. of bonds[b]
6-methyl	1.71	49.1	C5a	3
		70.9	C6	2
		116.7	C10a	4
		149.2	C6a	3
5a	2.66	24.6	C6-Me	3
		54.0	C4	3
		66.4	C11a	2
		204.9	C12	3
4a	3.22	49.1	C5a	3
		66.4	C11a	3
		88.3	C12a	2
		187.3	C1 or C3	3
		204.9	C12	3
4	4.19	49.1	C5a	3
		52.8	C4a	2
		66.4	C11a	2
		88.3	C12a	3
		98.8	C2	3
		204.9	C12	3
9	6.95	116.7	C10a	3
		149.2	C6a	4
		163.7	C10	2
7	7.19	70.9	C6	3
		116.67	C9	3
		116.70	C10a	3
8	7.62	116.67	C9	2
		116.70	C10a	4
		149.2	C6a	3

[a] LTc in acetone-d_6. [b] Indicates the number of bonds between the hydrogen and the carbon in the proposed LTc structure.

(ii) "Double Constant Time Pure Absorption Phase Heteronuclear Correlation (DOC)" Spectra.[32] This technique is also specific for proton/carbon coherence transfers over three bonds or less and the spectra, which show most of the same coherence transfers that were seen by INAPT, confirm those results. Specifically, the coherence transfers that involve three bonds or less, when one of those bonds is the postulated bridge between C-4 and C-11a, include H_{4a}/C-11a, H_{5a}/C-4, H_4/C-11a, and H_4/C-12. In addition, a hitherto unobserved coherence transfer between H_4 and the carbon signal at δ 96.9 now requires that this carbonyl carbon signal be due to C-3 or C-11, since C-1 is not within the requisite three-bond distance from H_4. Also, a coherence transfer is observed

(28) Advanced Varian XL-200 Manual (1986).

(29) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314–318.
(30) Bax, A.; Ferretti, N. N.; Jerina, D. M. *J. Org. Chem.* **1985**, *50*, 3029–3034.
(31) Bax, A. *J. Magn. Reson.* **1983**, *52*, 330–334.
(32) Jones, C. R.; Schoeder, S. A.; Gorenstein, D. G. *J. Magn. Reson.* **1988**, *80*, 370–374.

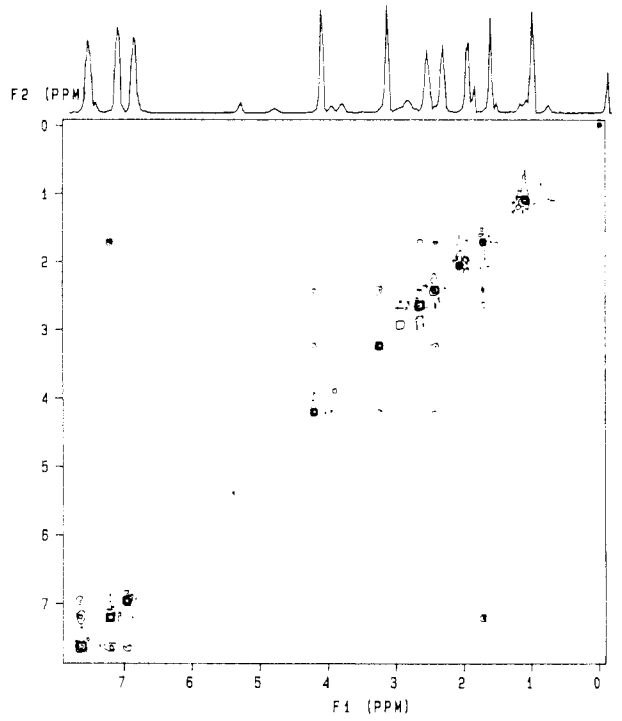

Figure 6. States pure absorption phase NOESY spectrum of LTc in acetone-d_6.

between H_{4a} and the carbon signal at δ 186.8, which now requires that the δ 186.8 signal be assigned to C-1 or C-3.

(iii) States Pure Absorption Phase NOESY Spectrum.[33] Several NOESY cross peaks can be seen in this spectrum (Figure 6). Such peaks can be recognized both by their frequency coordinates and by the fact that they are of negative sign relative to the diagonal. This has to be the case for authentic NOE's of small molecules using a pure absorption phase sequence. The most important result is the NOESY cross peak observed for H_4 and the H_5 pair of protons. The presence of this cross peak is consistent with the close proximity of these groups in the LTc structure. Other noteworthy cross peaks include H_4 to H_{4a}, H_5 to C_6-CH_3, H_{5a} to C_6-CH_3, H_7 to C_6-CH_3, and H_{4a} to H_5. The H_7 to H_7 and H_8 to H_9 cross peaks provide further support for the assignments in Table IV, as does a C_{10}-OH to H_9 cross peak at δ 12.3/δ 6.95 which is not shown.[34]

Summary of the NMR Data. An analysis of the LTc data indicates that most nuclei have resonances with the same chemical shift as they have in Tc, with only the H_4, C-4, and C-11a resonances showing substantial chemical shift changes, the latter presumably the result of a major chemical change at these sites. The COSY and HETCOR measurements confirm that there are two groups of scalar coupled protons, one group consisting of the three aromatic protons which are clearly sequential (strong scalar coupling of H_9 to H_8 to H_7) and thus on adjacent carbons, and a second group (H_{5a} to H_5 to H_{4a} to H_4) also bonded to adjacent carbons. The NOESY data show that the protons

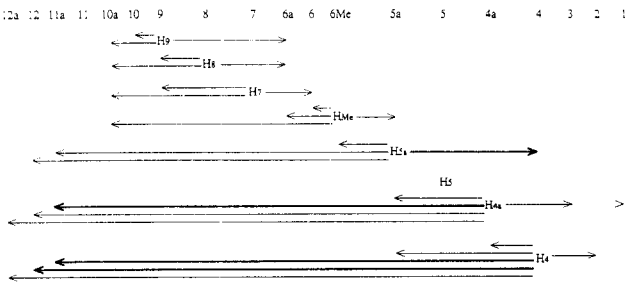

Figure 7. Diagrammatic representation of the proton to carbon long-range connectivities from the INAPT and DOC spectra of LTc. Connectivities involving the new C-4 to C-11a bond are made bold.

within each group are spatially close (strings of NOE's from H_7 to H_8 to H_9 and H_4 to H_{4a} to H_5 to H_{5a}), but the additional NOE's to the methyl protons confirm that both groups are immediately adjacent to this group. Thus, spatially, all of the nonexchangeable protons in the molecule are in one long string, with the H_4 to H_5 NOE's indicating that one end of the string is looped back on itself (see below). Most of the long-range proton to carbon connectivities obtained from the INAPT and DOC measurements are represented in Figure 7. Note again that the connectivities involving the methyl protons link together the aromatic and aliphatic strings of nuclei, as would be the case in Tc itself. However, the bolded arrows represent connectivities unique to LTc, and the H_{5a} to C-4, H_{4a} to C-11a, H_4 to C-11a, and H_4 to C-12 connectivities can only be explained by a new carbon–carbon bond between C-4 and C-11a. Furthermore, the folding of the molecule caused by this bond brings the H_4 proton into close proximity to H_5, in agreement with the H_4 to H_5 NOE observed in the NOESY spectrum. In summary, the NMR data conclusively show that LTc has the same carbon skeleton as Tc plus a new C-4 to C-11a carbon–carbon bond.

Conclusions. Lumitetracycline is a principal photoproduct when tetracycline is photolyzed with long-wavelength UV light in the absence of oxygen. There is preliminary evidence that this compound is itself phototoxic and that other Tc derivatives form analogous bridged products. Further studies directed toward an elucidation of the mechanistic details of LTc formation and a complete evaluation of its phototoxic properties are in progress.

Experimental Section

Chemicals. Tetracycline and tetracycline hydrochloride (Sigma) were used without further purification. Anhydrotetracycline was prepared from tetracycline.[35] The water used was distilled deionized water (Corning Megapure System). The following chemicals were used as received: acetonitrile and methanol (Burdick and Jackson, high purity); acetonitrile-d_3 (96% D or "100%" D), acetone-d_6 (98% D or "100%" D), DMSO-d_6 (99.5% D), and methanol-d_4 ("100%" D) (Aldrich or CIL). Other chemicals used were reagent grade.

Chromatography. HPLC employed a Varian 5000 series chromatograph with binary mixing capacity, fitted with a Rheodyne 7125 injection port. The detector was a Varian UV-100 UV absorbance detector, with either a Hewlett-Packard 3390 or a 3393A recording integrator for quantitative studies. The column used was an Alltech Econosil C-18 (4.6 mm × 25 cm, 10 μm particle size, initially passivated by flushing overnight with a 10 mM EDTA solution. A linear gradient was used which consisted of two eluents: (1) 30% v/v MeOH and 70% v/v buffer and (2) 3% MeCN, 75% MeOH, and 22% buffer, where "buffer" refers to

(33) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.

(34) Also seen in the full spectrum is an apparent cross peak between H_{10} and H_5 which is an artifact. It is exactly on the antidiagonal and if two diagonal peaks are symmetric with respect to the transmitter, as is the case here, than their image on the antidiagonal will not be eliminated by symmetrization. The C_6-OH and C_{12}-OH groups also give cross peaks with each other and with an impurity, probably H_2O. These cross peaks have the same sign as the diagonal (an indication that they are due to magnetization transfer by chemical exchange) and are not plotted in Figure 3.

(35) Simmons, D. L.; Woo, H. S. L.; Koorengevel, C. M.; Seers, P. *J. Pharm. Sci.* **1966**, *55*, 1313–1315.

Chart I

^1H	RD	s90	D6	s90s90	D6	s45s45	D1	s90s90	D7	decouple
^{13}C				180		90		180		acquisition

Chart II

A:	^1H	90°	evolution	90°	no decoupling			
	^{13}C			90°	acquisition			
B:	^1H	90°	evolution	90°	RD/2	s180°	RD/2	decoupling
	^{13}C			90°		180°		acquisition

a 500-mL aqueous solution containing 2% v/v acetic acid, 1% v/v triethylamine, and 3 mM EDTA (0.56 gm), final pH 3.5. A linear gradient was run from eluent 1 to eluent 2 over a period of 40 min at a flow rate of 1 mL/min, followed by eluent 2 for 15 min. The column was flushed with at least 10 mL of eluent 1 between runs. The retention times for Tc and its derivatives are as follows: Tc, 9 min; LTc, 17 min; DTc, 26 min; ATc, 33 min.

Preparative chromatography utilized a Bioanalytical Systems Miniprep 1200 system, with an ISCO 1840 UV-vis absorbance detector and recorder and an E. M. Merck Lichrosorb RP-8 (size B, 43–60 μm) LoBar column. Eluent conditions were either method A (30% v/v EtOH, 70% 50 mM oxalic acid (pH 3.0), flow rate 3 mL/min, monitored at 300 or 400 nm) or method B (10% v/v acetonitrile, 30% MeOH and 60% 0.2 M NH_4OAc (pH 7), flow rate 6 mL/min, monitored at 272 nm).

Spectroscopy. Ultraviolet absorption spectra were recorded using a Cary Model 17D, a Hewlett-Packard 8451A diode array, or a Perkin-Elmer Lambda 3B UV-vis spectrophotometer. Mass spectra were obtained on a Kratos MS-50 using positive chemical ionization (+CI, 20 eV) and fass atom bombardment (FAB, 20 eV).

NMR spectra were obtained by dissolving Tc or LTc in deuterated methanol, acetonitrile, acetone, or DMSO. The proton NMR spectra of Tc and LTc in acetone-d_6 (5 mg/mL) were recorded using the Nicolet 470-MHz instrument with the acetone δ 2.04 resonance as reference. The proton decoupled 1D carbon NMR spectra of Tc (100 mg/mL in acetone-d_6 (with trace amounts DMSO-d_6 used to predissolve the Tc) and in DMSO-d_6 were obtained on a Varian XL-200 (50 MHz) with TMS as reference. The proton-decoupled 1D carbon NMR spectra of LTc (200 mg/mL) were also obtained on a Varian XL-200, in acetone-d_6 and in acetonitrile-d_3, with TMS as reference. The Varian XL-200 was also used for the fully coupled carbon NMR spectrum and the "attached proton test" of the carbon NMR of LTc (200 mg/mL), the homonuclear experiment on LTc (200 mg/mL), and the heteronuclear correlation experiment on Tc (100 mg/mL) and LTc (200 mg/mL), all in acetone-d_6.

The INAPT spectra were obtained on a Nicolet NT-300 (proton frequency 300 MHz) in acetone-d_6 with the sequence[29,30] and parameters shown in Chart I, where the 90 and 180 carbon pulses required 13 and 26 μs, the selective 90 and 45 proton pulses s90 and s45 were 6.50 and 3.25 ms in length, the recycle delay RD was 5 s, and the rest of the delays were D1 = 43.5 ms, D6 = 40.25 ms, and D7 = 47.80 ms.

Two versions of the DOC technique[32] were used to obtain these spectra on an XL200 Advanced spectrometer (see Chart II), where evolution is

^1H	CD-t1/4	180°	t1/4	s180°	CD-t1/4	180°	t1/4
^{13}C		180°		180°		180°	

and where CD is the constant delay (the total evolution delay is 2 × CD), RD is the refocusing delay, and s180 is a semiselective proton inversion produced by a DANTE train of twenty pulses separated by the DANTE delay DD. DOC sequences produce a $^1H/^{13}C$ 2D spectrum which only shows correlations between protons in the region inverted by the semi-selective pulse and nearby carbons. Spectra were taken with the selective pulse inverting single protons, sequentially H_4, H_{4a}, and H_{5a}. Sequence A was used, with CD = 45 and DD = 0.3 ms, for coherence transfer from the H_4 proton sequence. For coherence transfers from H_{4a}, two DOC spectra were obtained, one with sequence A with CD = 40.5 and DD = 0.6 ms, and the other using sequence B with CD = 30, RD = 50, and DD = 0.6 ms. For coherence transfers from H_{5a}, two DOC spectra were obtained, one using sequence A with CD = 40.5 and DD = 0.85 ms, and the second using sequence B with CD = 40.5, RD = 50, and DD = 0.85 ms.

The States pure absorption phase NOESY spectrum was obtained in acetone-d_6 on a Varian Germini 200 spectrometer with a mixing time of 1 s, a recycle delay of 4 s, and a 3000-Hz spectral window in both dimensions. Two data fields, each consisting of 256 FID's with 704 data points in each FID, were processed with a hypercomplex transform.[33]

The simulation of the resolution-enhanced (EM = −0.34) proton NMR spectrum of LTc in methanol-d_4 was run on a Varian XL-200 using an iterative programs based on the Fortran program LAME.[28]

Photolyses. Irradiations were done with a Canrad-Hanovia 450-W medium pressure mercury arc lamp filtered through uranium yellow glass (λ > 330 nm, Houde Glass). The lamp and filter sat within an Ace Glass immersion well which was at the center of a turntable (for photolyses in tubes) or inserted in a reaction vessel (for preparative runs). The photolyses were carried out in a water bath, with the temperature maintained using a Neslab RTE-3 or RTE-4 circulating cooling unit. Argon degassing was done through polyethylene tubing (0.58 mm i.d. Clay Adams).

Preparation and Isolation of LTc. Tc (120 mg) was dissolved in ca. 125 mL of acetonitrile containing 120 μL of acetic acid, placed in the reaction vessel, deoxygenated by bubbling with Ar for 15 min, and irradiated for 13 h with continuous Ar bubbling. The initial workup consisted of adding ca. 50 mL of water (the water was in all cases purified as noted in the Chemicals Section) and 5 mL of 4 N HCl to the photolysate and concentrating the solution under vacuum (T < 40 °C) to ca. 60 mL. Alternatively, Tc·HCl (120 mg) in 0.1 M phosphate buffer (125 mL) was deoxygenated and irradiated for 10 h (continuous Ar bubbling) using an initial acidification with 10 mL of 4 N HCl as the initial workup. The acidified aqueous solutions from the initial workups were then treated similarly. Approximately 5–10 mL of brine was added to the solutions, and they were extracted with EtOAc (4 × 20 mL). The extracts were combined, washed with 25 mL of H_2O, dried over anhydrous Na_2SO_4, filtered through glass wool, and evaporated to dryness in vacuo (T < 35 °C).

The crude LTc mixtures were purified using preparative reverse-phase chromatography by dissolving the crude mixture in 2–3 mL of MeOH and diluting to twice the volume with the buffer component of the eluent used. The samples were loaded on to the column in 1–1.5-mL increments and injected over a period of up to 5 min. Samples from the photolyses in acetonitrile were purified by both methods A and B while only method B was used on for the product formed in phosphate buffer. With method A, LTc elutes at 144–204 mL, between Tc (96–144 mL) and dedimethylaminotetracycline (230–300 mL). Residual oxalic acid from the eluent can be seen in some of the NMR traces of LTc isolated in this fashion. With method B, LTc is the first major peak off the column, eluting at 70 mL to ca. 220 mL (solvent front ca. 50 mL).

After chromatography, the LTc samples were acidified by the addition of several milliliters of 4 N HCl and evaporated in vacuo (T < 40 °C) to 40–50% of the original volumes. The solutions were then extracted 4 times with 15–20-mL fractions of EtOAc, and the combined extracts were washed once with 20 mL of H_2O and dried over anhydrous Na_2SO_4. A pinch of carbon black was added, and the solution was filtered through additional drying agent using a medium frit glass funnel and then reduced to 3–4 mL in vacuo (T < 35 °C). Approximately 150–300 mL of hexanes

was added to precipitate LTc as a flocculent off-white solid. The LTc was collected by vacuum filtration through a 13-mm Nylon 66 0.45-μm membrane (Alltech) with air drawn through the precipitate to dry it. The sample was then placed under vacuum (<1 mmHg) overnight. This procedure afforded yields of up to 50% for runs in acetonitrile (with acetic acid) and up to 70% yields for runs in phosphate buffer.

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Registry No. Tc, 60-54-8; Tc·HCl, 64-75-5; LTc, 115747-16-5.

Intramolecular [4 + 2]-Cycloaddition Chemistry of Some 1,3-Dienyl-Substituted Cyclopropenes

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The thermal reaction of several 1,3-dienyl-substituted diphenylcyclopropenes has been studied. Thermolysis of these systems give products derived from an intramolecular [4 + 2]-cycloaddition. The reaction involves bond formation between the dienyl π-bond and the cyclopropene to produce a diradical intermediate which collapses to the observed product. In addition to the [4 + 2]-cycloadduct, another compound formed corresponds to the [2 + 2]-adduct. During the course of our studies with (triphenylcyclopropenyl)cyclopentadiene, we found that variously substituted cyclopropenes react with 4-phenyl-1,2,4-triazoline-3,5-dione to give rearranged urazole derivatives. The reaction involves electrophilic addition of PTAD on the reactive π-bond to give an aziridinium ion which undergoes facile rearrangement followed by dipole collapse to produce the urazole. The bimolecular Diels–Alder reaction of 1-phenyl-2-vinyl-3,3-dimethylcyclopropene with *N*-methyl-1,2,4-triazoline-3,5-dione was also examined and was found to afford a novel rearranged cycloadduct.

The chemistry of cyclopropene derivatives has attracted considerable interest mainly because of the high strain energy in the ground state associated with the unsaturated three-membered ring.[1-10] One of the ways of relieving bond angle strain in cyclopropenes involves [4 + 2]-cycloaddition across the reactive π-bond.[11] With the notable exception[12,13] of molecules containing bulky groups at C_3, cyclopropenes readily undergo Diels–Alder cycloaddition to give bicyclo[4.1.0]hept-3-enes in good yield.[14] The bimolecular [4 + 2]-reaction of substituted cyclopropenes is usually subject to a strong steric preference for an exo transition state leading to the Diels–Alder cycloadduct.[3,15] The preference for exo approach is the result of an unfavorable steric interaction of the geminal substituents in the 3-position with the diene in the endo transition state. Only in instances in which the endo

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