Table I. Single-Crystal X-ray Crystallographic Analysis

A. Crystal Parameters	
formula	$C_{10}H_{13}IO_3$ (308.11)
crystallization medium	isopropyl ether
crystal size, mm	0.27×0.28×0.38
cell dimensions	
a, Å	6.748 (1)
b, Å	11.258 (2)
c, Å	14.333 (2)
α, deg	90.0
β, deg	90.0
γ, deg	90.0
V, Å^3	1088.8 (3)
space group	$P2_12_12_1$
molecules/unit cell	4
density obsd, g/cm^3	1.87
density calcd, g/cm^3	1.879
linear absorption coefficient, cm^{-1}	29.6

B. Refinement Parameters	
number of reflections	711
non-zero reflections ($I > 1.0\sigma$)	705
R index=$\Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0.040
GOF = $[\Sigma w(F_o^2 - F_c^2)^2/(m - S)]^{1/2}$	3.77
scale factor	0.814 (7)
secondary extinction coefficient	16.1 (6) × 10^{-6}

were all zero. The final R index was 0.040. A final difference Fourier revealed no missing or misplaced electron density. The absolute configuration of the molecule was determined by the method of Ibers and Hamilton.[15] The refined structure was plotted by using the ORTEP computer program of Johnson[16] (Figure 1). This configuration was established as correct at the 0.5% level of significance (i.e., with 99.5% confidence).[17]

Tables of coordinates, anisotropic temperature factors, distances, and angles are available as supplementary material from J. B.

(2*S*,4*S*)-Ethyl 1-Methoxybicyclo[2.2.2]oct-5-ene-2-*endo*-carboxylate ((*S*)-6) and (2*R*,4*R*)-Ethyl 1-Methoxybicyclo-[2.2.2]oct-5-ene-2-*endo*-carboxylate ((*R*)-6). The resolved acids (*S*)-5 and (*R*)-5 were esterified in refluxing ethanol with catalytic *p*-toluenesulfonic acid. (*S*)-5, (–) endo acid (15 g), gave ethyl ester (*S*)-6 (14.5 g) as an oil in 86% yield: bp 100–103 °C (0.4 mm); $[\alpha]_D$ –5.08° (*c* 1.103, $CHCl_3$); NMR (250 MHz) δ 6.25 (m, 2), 4.12 (m, 2), 3.38 (s, 3), 2.89 (q, 1), 2.55 (bs, 1), 1.88 (m, 1), 1.24 (t, 3). Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.57; H, 8.57. Found: C, 68.66; H, 8.53.

(*R*)-5, (+) endo acid (22.8 g), gave ethyl ester (*R*)-6 (22.9 g) as an oil in 87% yield: bp 98–102 °C (0.3 mm); $[\alpha_D]$ +6.18° (*c* 1.1, $CHCl_3$); IR (CH_2Cl_2) 1730 cm^{-1}; mass spectrum, *m/e* 196 (M$^+$).

(2*S*,4*S*)-Ethyl 1-Hydroxybicyclo[2.2.2]oct-5-ene-2-*endo*-carboxylate ((*S*)-7) and (2*R*,4*R*)-Ethyl 1-Hydroxybicyclo-[2.2.2]oct-5-ene-2-*endo*-carboxylate ((*R*)-7). The ester (*R*)-6 (21 g, 0.1 mol) in CH_2Cl_2 (250 mL) at –25 °C was treated dropwise with 1 M BBr_3 (110 mL). After 1 hour of stirring, the reaction was quenched into cold saturated aqueous $NaHCO_3$. The desired alcohol (*R*)-7 was isolated from CH_2Cl_2 and distilled in vacuo: 17.7 g, 90%: bp 88–90 °C (0.25 mm); $[\alpha]_D$ –37.5° (*c* 1.12, $CHCl_3$); IR (CH_2Cl_2) 3547 (OH), 1710 cm^{-1}; NMR (250 MHz) δ 6.18 (d, 2), 4.12 (q over bs, 3), 2.69 (q, 1), 2.55 (bs, 1), 1.96 (m, 1), 1.24 (t, 3); mass spectrum, *m/e* 196 (M$^+$). Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.30; H, 8.16. Found: C, 67.04; H, 8.09.

The (*S*)-6 ester (4 g, 0.019 mol) was treated with 1 M BBr_3 (20 mL), giving the tertiary alcohol (*S*)-7, 3.5 g, 95% yield: bp 85–87 °C (0.2 mm); $[\alpha]_D$ +38.24° (*c* 1.13, $CHCl_3$); IR (CH_2Cl_2) 3546 (OH), 1729/1709 cm^{-1}; mass spectrum, *m/e* 196 (M$^+$).

(*R*)-Ethyl 3-(4-Oxocyclohex-2-enyl)propionate ((*R*)-1) and (*S*)-Ethyl 3-(4-Oxocyclohex-2-enyl)propionate ((*S*)-1). (–)-Ethyl-1-hydroxybicyclo[2.2.2]oct-5-ene-2-*endo*-carboxylate ((*R*)-7) (16.5 g, 0.079 mol) in *tert*-butyl alcohol (165 mL) was treated with *t*-BuOK (0.44g, 0.0039 mol) at room temperature for 45 min. The reaction was diluted with EtOAc and washed with pH 6.0 phosphate buffer (2 × 100 mL), water, and brine. The desired product (*R*)-1 was recovered from the organic layer and distilled in vacuo, giving a clear liquid; 14.5 g, 88% yield: bp 100–105 °C (0.25 mm); $[\alpha]_D$ –81.9° (*c* 1.15, $CHCl_3$); IR (CH_2Cl_2) 1731 (s), 1678 (s) cm^{-1}; mass spectrum, *m/e* 197 (M$^+$ + 1), 196 (M$^+$).

(*S*)-7 (3 g, 0.015 mol) gave the cyclohexenone (*S*)-1 (2.6 g, 90%) with *t*-BuOK (85 mg, 0.0008 mol) in *tert*-butyl alcohol (25 mL): bp 103–108 °C (0.4 mm); $[\alpha]_D$ +85.5° (*c* 1.115, $CHCl_3$); IR (CH_2Cl_2) 1730, 1680 cm^{-1}; NMR (250 MHz) δ 6.83 (dq, 1), 6.0 (dd, 1), 4.15 (q, 2), 1.26 (t, 3); mass spectrum, *m/e* 197 (M$^+$ + 1), 196 (M$^+$). Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.30; H, 8.16. Found: C, 67.36; H, 8.06.

(2*R*,4*S*)-(–)-1-Methoxybicyclo[2.2.2]oct-5-ene-2-*exo*-carboxylic Acid ((*S*)-4). Racemic exo acid *rac*-4 (55 g, 0.3 mol) and *l*-ephedrine (49.9 g, 0.3 mol) gave a crystalline salt from refluxing ethyl acetate (350 mL) upon cooling to room temperature. This was recrystallized once from ethyl acetate, giving the (*S*)-4 salt: 29.3 g, 28%; mp 135–136 °C; $[\alpha]_D$ –40.7° (*c* 1.156, MeOH). Anal. Calcd for $C_{20}H_{29}NO_4$: C, 69.14; H, 8.41; N, 4.03. Found: C, 69.15; H, 8.39; N, 4.32

This salt (5 g, 0.014 mol) was converted to the free acid (*S*)-4 (2.2 g, 88%) as described: mp 78–81 °C; $[\alpha]_D$ –109.1° (*c* 1.245, CH_2Cl_2); NMR identical with racemate. Anal. Calcd for $C_{10}H_{14}O_3$: C, 66.00; H, 7.75. Found: C, 65.66; H, 7.66.

Acknowledgment. We thank Professor E. J. Corey of Harvard University for useful discussions during the course of this work.

Registry No. (*S*)-1, 94050-15-4; (*R*)-1, 95782-19-7; (±)-3, 95782-20-0; (*S*)-3, 95783-35-0; (±)-4, 95782-21-1; (*S*)-4 ((–)-α-methoxy-α-(trifluoromethyl)phenylacetate), 95694-09-0; (*S*)-4·*l*-ephedrine, 94246-61-4; (*S*)-4, 94198-69-3; (±)-5, 95782-22-2; (*S*)-5, 94198-70-6; (*R*)-5, 95782-23-3; (*S*)-5·*d*-ephedrine, 94246-60-3; (*R*)-5·*l*-ephedrine, 95839-07-9; (*S*)-5 ((–)-α-methoxy-α-(trifluoromethyl)phenylacetate), 95782-24-4; (*R*)-5 ((–)-α-methoxy-α-(trifluoromethyl)phenylacetate, 95782-25-5; (*S*)-6, 94132-10-2; (*R*)-6, 95782-26-6; (*S*)-7, 94132-11-3; (*R*)-7, 95782-27-7; (–)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 39637-99-5.

(15) Ibers, J.; Hamilton, W. C. *Acta Crystallogr.* **1964**, *17*, 781.
(16) Johnson, C. K. ORTEP Report ORNL-3794, 1965, Oak Ridge National Laboratory, TN.
(17) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.

Anhydrotetracycline is a Major Product of Tetracycline Photolysis

Tayyaba Hasan,† Mark Allen, and Barry S. Cooperman*

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received August 7, 1984

Tetracycline (TC) is a low molecular weight, broad-spectrum antibiotic that inhibits protein synthesis by preventing the binding of aminoacyl-tRNA to the A site of ribosomes.[1] Its photochemistry is of direct importance to prior photoaffinity labeling studies of this group aimed at identifying the site of TC binding to the Escherichia coli ribosome.[2] We found that even in the presence of β-mercaptoethanol, the addition of which affords the most site-specific photoincorporation of TC, a TC photoproduct was formed that labels the ribosome in a nonspecific manner. This report describes the isolation and identification of 5a,6-anhydrotetracycline (AHTC) as the major product formed on photolysis of TC under the conditions of our photoaffinity labeling experiment. The formation of AHTC accounts not only for our prior results but also

† Current address: Photomedicine Research Unit, Department of Dermatology, Massachusetts General Hospital, Boston, MA 02114.

0022-3263/85/1950-1755$01.50/0

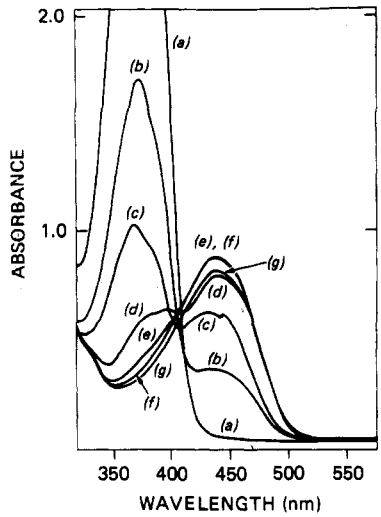


Figure 1. Time course of UV–vis spectrum of TC (94 μM) in TMK buffer (Tris·HCl, 50 mM, pH 7.6; $MgCl_2$, 10 mM; KCl, 50 mM) containing 0.1% v/v of β-mercaptoethanol, subjected to photolysis at 4 °C. Spectra were taken after photolysis for (a) 0 min; (b) 30 min; (c) 60 min; (d) 90 min; (e) 120 min; (f) 150 min; (g) 180 min. The initial A_{373} value was 2.84.

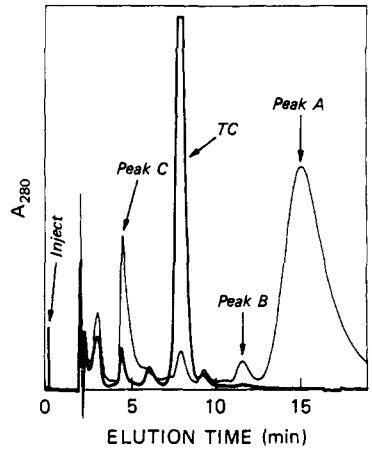
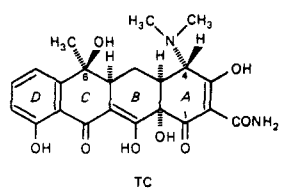
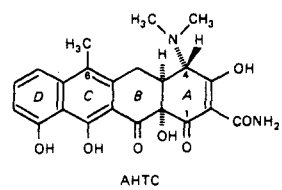


Figure 2. HPLC profile of TC photolysis mixture. Samples of a solution of TC in buffer (as described in the legend to Figure 1) both before (heavy line) and after (narrow line) photolysis (1.5 h) were concentrated by lyophilization and the concentrates were injected into a μBondapak (Waters) RPC_{18} silica gel column (10 μM silica gel, 100-Å pore size). The mobile phase was a solution containing 18 parts of dimethylformamide and 82 parts of an aqueous solution of oxalic acid (75 mM) and EDTA (free acid, 20 mM), brought to pH 6.4 with triethylamine. The flow rate was 1.4 mL/min.

suggests a mechanism for the cutaneous phototoxicity of TC.[3]





(1) Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. "The Molecular Basis of Antibiotic Action", 2nd ed.; Wiley: New York, 1981; pp 448–453.
(2) Goldman, R. A.; Hasan, T.; Hall, C. C.; Strycharz, W. A.; Cooperman, B. S. *Biochemistry* **1983**, *22*, 359.
(3) Hasan, T.; Kochevar, I. E.; McAuliffe, D. J.; Cooperman, B. S.; Abdulah, D. *J. Invest. Dermatol.* **1984**, *83*, 179.

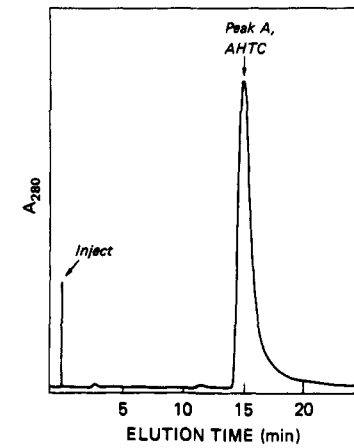


Figure 3. HPLC profile of a mixture of peak A and authentic AHTC. Peak A (Figure 2) was collected and mixed with authentic AHTC, and the resulting solution was subject to HPLC analysis as in Figure 2, except that the mobile phase was 27% CH_3CN in deionized water containing 0.1% CF_3CO_2H and the flow rate was 1.3 mL/min. Note that peak A and AHTC, when chromatographed separately, each gave a peak with the same retention time as that depicted.

Results and Discussion

Irradiation of TC in the presence of β-mercaptoethanol (0.1%) with 3500-Å lamps leads to a marked change in the UV–vis spectrum, characterized by a loss in peak intensity at 373 nm and the formation of a new peak at 438 nm (Figure 1). In the experiment shown, the half-life for this initial process is 40–45 min; more prolonged photolysis leads to destruction of the new chromophore at 438 nm. On the basis of these results, photolysis conditions were chosen to maximize absorption at 438 nm. High performance liquid chromatography (HPLC) of the reaction mixture before and after photolysis (Figure 2) shows the presence of a major product peak A, t_R 15.0 min, well resolved from residual TC (t_R 7.8 min), and two other minor new peaks, peak B, t_R 11.7 min, and peak C, t_R 4.5 min. Purified peak A, when frozen, is stable to storage indefinitely (as shown by HPLC analysis). However, when stored at room temperature for $\simeq$ 1 h it gives rise to small amounts of peak B.

That peak A is the major photoproduct of TC is shown not only by the HPLC analysis but also by the fact that its electronic absorption spectrum (λ_{max} 270 and 429 nm) is virtually identical with that of the photolysis reaction mixture. We have identified peak A as AHTC and peak B as 4-*epi*-anhydrotetracycline (E-AHTC) as described below. The structure corresponding to the minor product C has not yet been determined.

The identification of peaks A and B is based on the following evidence. (i) AHTC cochromatographs with peak A (Figure 3). Furthermore, when AHTC is incubated under conditions known to give rise to 4-*epi*-anhydrotetracycline (E-AHTC) and the resulting mixture is analyzed by HPLC, the E-AHTC peak comigrates with peak B. (ii) The UV–vis spectra of AHTC, E-AHTC, peak A, and peak B are all essentially identical. (iii) A sample of peak A, prepared for spectral analysis from a photolysis reaction mixture by HPLC separation (using the mobile phase described in the legend to Figure 3 which is fully volatile and easily removed by lyophilization), has a ^1H NMR spectrum in pyridine-d_5 that is essentially identical with that of AHTC, as described below (δ values in ppm from tetramethyl silane). Here it is particularly important to note the chemical shift of the C_6-methyl group, which at 2.4 ppm is 0.6 ppm further downfield than that of the

C_6-methyl group in TC, consistent with the aromatization of ring C. Similar spectra for peak A and AHTC were also

AHTC

2.39 (s, 3 H), 2.59 (s, 6 H), 3.15 (m, 1 H), 3.59 (m, 1 H), 3.63 (b, 1 H), 3.73 (d, 1 H)

peak A

2.40 (s, 3 H), 2.59 (s, 6 H), 3.12 (m, 1 H), 3.62 (b, 2 H), 3.7 (d, 1 H)

observed in MeOH-d_4. Typical yields of AHTC by HPLC recovered from the photolysis mixture ranged from 40% to 45%.

In previous studies of TC photochemistry, three different photoproducts have been reported,[4-6] although in no case has the identification been unequivocal. Thus, (a) Hlavka and Bitha[4] found that irradiation of TC in MeOH leads to dimethylamine formation and concluded that they had photoreductively deaminated TC at the 4 position, (b) Davies et al.[5] isolated a red product formed on irradiation of an air-saturated solution of TC at pH 9 and concluded on the basis of its chemical properties that it was a quinone formed on oxidation of photodeaminated TC at the 4 position, and (c) Sanniez and Pilpel[6] photolyzed TC in an oil–water mixture and identified AHTC as one of the principal products formed on the basis of TLC and UV spectroscopy, although little detail was provided. Given this rather complex background we thought it important to determine the TC photoproduct formed under the conditions of our photoaffinity labeling experiments. Our results present the first well-documented proof for the formation of AHTC on photolysis of TC under mildly reducing conditions (presence of β-mercaptoethanol). This photodehydration is undoubtedly driven by the aromatization of ring C. Such reactions are otherwise rather rare, although it is worth noting that ethylene glycol has been shown to photodehydrate to acetaldehyde in a reaction that, like the one described in this paper, requires the presence of SH compounds.[7]

Elsewhere we have shown that the identities of products produced on TC photolysis are strongly influenced by redox conditions (O_2 vs. N_2 atmosphere, presence or absence of β-mercaptoethanol).[2] We are currently seeking to determine whether the products formed in the absence of β-mercaptoethanol correspond to those put forward by earlier workers, as described above. If the singlet oxygen that is produced on photolysis of oxygen-containing solutions of TC[8] is a reactant in the formation of these products, then it is possible that β-mercaptoethanol exerts its marked effect on product formation by preventing the accumulation of singlet oxygen in solution. Such an explanation would also provide a rationale for the difference in the results obtained by Davies et al.[5] and by Sanniez and Pilpel[6] since in the latter study photolysis proceeds in the oil phase, away from the oxygen dissolved in the aqueous phase.

The formation of AHTC on TC photolysis is quite consistent with the photoaffinity labeling studies alluded to above, since AHTC is only a poor inhibitor of ribosomal function[9] and would not be expected to show strong site-specific binding. In addition, formation of AHTC, which has known cutaneous phototoxicity,[3] could account for TC-induced cutaneous phototoxicity.

Experimental Section

HPLC analysis was performed on a Waters Associates chromatograph consisting of 6000A and M-45 pumps, a 660 programmer, and a U6K Universal injector. The detection system was a Waters extended wavelength module (214 nm) and a Model 440 absorbance detector connected in series. UV–vis spectra were recorded on a Beckman D-8 spectrophotometer. NMR spectra were obtained on either an IBM-200 FT-NMR or a Bruker 250-MHz FT NMR. Photolysis experiments were carried out in 100-mL round-bottomed Pyrex flasks with either Rayonet RPR 3500-Å lamps or with a UV Products, Inc., PCQ 008L lamp assembly having a maximal output between 3400 and 3800 Å.

Tetracycline was obtained from Lederle Laboratories and was ≳98% pure. It was used without further purification. AHTC was prepared by acid-catalyzed dehydration of TC.[10] Deuterated solvents were obtained from Aldrich Chemical Company; both the methanol and pyridine used in the study had 99.9 atom % D. All other chemicals were of the highest grade of purity available.

Acknowledgment. This work was supported by NSF grants to B.S.C. (PCM81-04360 and PCM84-10152). Support for T.H. from the A.O. and G.N. Wellman Foundation is also gratefully acknowledged.

Registry No. AHTC, 1665-56-1; TC, 60-54-8.

(4) Hlavka, J. J.; Bitha, P. *Tetrahedron Lett.* **1966**, 3843.
(5) Davies, A. K.; McKellar, J. F.; Phillips, G. O.; Reid, A. G. *J. Chem. Soc., Perkin Trans. 2* **1979**, 369.
(6) Sanniez, W. H. K.; Pilpel, N. *J. Pharm. Sci.* **1980**, *69*, 5.
(7) Rudakova, I. P.; Ershova, T. E.; Belikov, A. B.; Yurkevich, A. M. *J. Chem. Soc., Chem. Commun.* **1978**, 592.
(8) Wiebe, J. A.; Moore, D. E. *J. Pharm. Sci.* **1977**, *66*, 186.
(9) Summ, H. D.; Christ, O. *Arzneim-Forsch.* **1967**, *17*, 1186.
(10) Green, A.; Wilkinson, R. G.; Booth, J. H. *J. Am. Chem. Soc.* **1960**, *82*, 3946.

Nitrogen-15 and Phosphorus-31 NMR Spectroscopy of *N*-Aryl-*P*,*P*,*P*-triphenylphospha-λ⁵-azenes. Applicability to pπ–dπ Bonding

Martin Pomerantz,*[1a] Barbara T. Ziemnicka,[1a,b] Zohar M. Merchant,[1a] Whe-Narn Chou,[1a] Wesley B. Perkins,[1a] and Shmuel Bittner[1c]

Department of Chemistry, The University of Texas at Arlington, Arlington, Texas 76019-0065, and Department of Chemistry, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel

Received September 18, 1984

The concept of pπ–dπ overlap has long been employed to explain observations involving molecules containing a third-row element such as phosphorus bound to a second-row element such as nitrogen or oxygen containing nonbonding electrons.[2] One such system is the phospha-λ⁵-azene, shown below as a resonance hybrid of structures A and B.

$$\underset{\text{A}}{R_3\overset{+}{P}\overset{-}{N}R'} \longleftrightarrow \underset{\text{B}}{R_3P{=}NR'}$$

The numerous reports which claim that the ^{31}P NMR chemical shifts of phospha-λ⁵-azenes, particularly of the type 1, are sensitive to the contribution of the pπ–dπ resonance form to the hybrid,[3-5] and a very recent com-

(1) (a) The University of Texas at Arlington. (b) On leave from the Institute of Physics, Technical University of Lodz, Poland. (c) Ben-Gurion University of the Negev.
(2) Kwart, H.; King, K. G. "d-Orbitals in the Chemistry of Silicon, Phosphorus and Sulfur"; Springer-Verlag: Berlin, 1977. Allcock, H. R. "Phosphorus–Nitrogen Compounds"; Academic Press: New York, 1972; Chapter 3.

0022-3263/85/1950-1757$01.50/0