30-mg samples by the Faraday method. Between 77 and 300°K, the susceptibility follows closely the Curie–Weiss law, $\chi_m = C_m/(T - \Theta)$, with $\Theta = -20°$ for I and $\Theta = -12°$ for II. At 292°K, the magnetic moments found are 3.98 ± 0.05 BM for I and 3.80 ± 0.05 BM for II. In 0.05 *M* methanolic solution, moments of 3.80 ± 0.15 BM for I[4] and 3.82 ± 0.15 BM for II were measured by the Gouy method. The agreement between the measurements on solid samples and in solution as well as the rather small Θ values rule out the possibility of a spin-exchange interaction between different iron(II) ions at ambient temperature. The magnetic moments thus indicate an $S = 1$ electronic ground state. As in square-planar compounds,[1,2] an orbital contribution of ~1.0 BM has to be assumed, the source of which is not obvious on the basis of simple ligand field theory.[5]

Studies of the ^{57}Fe Mössbauer effect at 293°K using the coupled-loudspeaker technique result in isomeric shifts[6] $\delta = 0.31 \pm 0.05$ mm/sec for I and $\delta = 0.32 \pm 0.04$ mm/sec for II. Quadrupole splittings were determined as $\Delta E_Q = 0.21 \pm 0.03$ mm/sec for I and $\Delta E_Q = 0.18 \pm 0.02$ mm/sec for II. The same values within experimental uncertainties were obtained at 77°K.[7] These values differ considerably from those measured on $[Fe(phen)_2X_2]$ compounds having $S = 2$ ground states ($^5T_2$ in strict O_h symmetry[8]). In this case, ΔE_Q values between 2.68 and 3.00 mm/sec are found, whereas δ varies between 0.96 and 1.13 mm/sec.[7] Also, the Mössbauer spectra of I and II do not conform with spectra of those compounds within the same series which exist in thermal equilibria between almost equienergetic $^5T_2$ and $^1A_1$ ground states.[9,10] The possibility that the increased magnetic moments might arise by analogous quintet–triplet equilibria is ruled out simultaneously. The spectra of I and II are rather similar to the Mössbauer spectra of compounds in $^1A_1$ ground states like $[Fe(phen)_2(CN)_2]\cdot H_2O$, where, at 293°K, $\Delta E_Q = 0.59$ mm/sec and $\delta = 0.22$ mm/sec,[11] although there is a significant difference in that the ΔE_Q values are definitely smaller. Thus, it seems justified to assume that the Mössbauer parameters presented here are characteristic for six-coordinated iron(II) in an $S = 1$ ground state.

The infrared spectra of I and II, which were studied between 4000 and 400 cm^{-1}, show the frequencies ν_1, ν_2, ν_3, ν_4, ν_7, ν_8, ν_9 of the coordinated oxalate and malonate ion.[12] As far as the frequencies of the phenanthroline ligand are concerned, it has been shown[13] that there are significant differences in the infrared between compounds in the $^5T_2$ and $^1A_1$ ground states, these differences being related to metal–ligand distances and bonding. In this regard, the spectra of I and II compare well with those of compounds in $^1A_1$ states. This is demonstrated, *e.g.*, by the C=C and C=N stretching vibrations (very weak bands at 1518, 1514, and 1493 cm^{-1}; strong bands at 1429 and 1413 cm^{-1}) and the α(CCC) mode (561 and 532 cm^{-1}). The bands at 1103 and 867 cm^{-1}, which are characteristic of the $^5T_2$ state, are not observed. Thus, in compounds having $S = 1$ ground states, distances and bonding between the metal ion and the phenanthroline ligands seem to have definite similarities to compounds in $^1A_1$ rather than to those in $^5T_2$ ground states. This inference which is supported by the Mössbauer data is also consistent with results from electronic spectra.

Compound I was precipitated by acetone from an aqueous solution of $[Fe(phen)_3]C_2O_4$ which was obtained from FeC_2O_4 and 1,10-phenanthroline (*Anal.* Calcd: C, 52.54; H, 4.41; N, 9.43; Fe, 9.40; H_2O, 15.13. Found: C, 52.68; H, 4.28; N, 9.42; Fe, 9.55; H_2O, 15.29). Compound II was prepared analogously from $[Fe(phen)_3]CH_2C_2O_4$ which was obtained starting from $FeCH_2C_2O_4\cdot py\cdot H_2O$ (*Anal.* Calcd: C, 50.32; H, 5.01; N, 8.70; O, 27.32; Fe, 8.65; H_2O, 19.57. Found: C, 50.25; H, 5.04; N, 8.65; O, 27.58; Fe, 8.84; H_2O, 19.33). All operations were performed under nitrogen. The compounds are of *cis* configuration, monomeric, and nonelectrolytes (molar conductivity 15.2 and 21.6 mho cm^2/mole in 0.01 *M* methanolic solution at 298°K for I and II, respectively). The detailed preparation procedure and chemical characterization will be reported in a forthcoming publication.[14]

(4) The lower moment obtained for I in solution seems to be due to slight decomposition.
(5) B. N. Figgis and J. Lewis, *Progr. Inorg. Chem.*, **6**, 37 (1964).
(6) The isomeric shifts, δ, are measured relative to the midpoint of the spectrum of an iron-foil absorber. A source of ^{57}Co diffused into stainless steel has been used.
(7) S. Hüfner, E. Steichele, E. König, and K. Madeja, *Z. Naturforsch.*, in press.
(8) For convenience, the notation of O_h symmetry will be used.
(9) E. König and K. Madeja, *Chem. Commun.*, 61 (1966).
(10) E. König and K. Madeja, submitted for publication.
(11) For additional examples, *cf.* ref 7.
(12) K. Nakamoto, "Infrared Spectra of Inorganic and Coordination Compounds," John Wiley and Sons, New York, N. Y., 1963.
(13) E. König and K. Madeja, *Spectrochim. Acta*, in press.
(14) K. Madeja, to be published.
(15) North Atlantic Treaty Organization Research Fellow, 1966.
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Oxidative Preparation of 9-Hydroxytetracyclines

Sir:

Methods for hydroxylation of certain tetracyclines at positions 5, 6, and 12a have been disclosed by Holmlund, *et al.*,[1,2] and Miller, *et al.*[3,4] Hlavka, *et al.*,[5] were also able to prepare biologically active 7-hydroxy-6-demethyl-6-deoxytetracycline using a procedure which is limited to modification of 6-demethyl-6-deoxytetracycline.

While exploring alternative hydroxylation methods, we have examined the Udenfriend reagent which has been shown to oxidize a large number of biological compounds. In particular, the hydroxylation of aro-

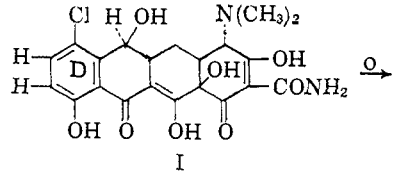
(1) C. E. Holmlund, W. W. Andres, and A. J. Shay, *J. Am. Chem. Soc.*, **81**, 4748 (1959).
(2) C. E. Holmlund, W. W. Andres, and A. J. Shay, *ibid.*, **81**, 4750 (1959).
(3) P. A. Miller, A. Saturnelli, J. H. Martin, L. A. Mitscher, and N. Bohonos, *Biochem. Biophys. Res. Commun.*, **16**, 285 (1964).
(4) P. A. Miller, J. H. Hash, M. Lincks, and N. Bohonos, *ibid.*, **18**, 325 (1965).
(5) J. J. Hlavka, H. Krazinski, and J. H. Boothe, *J. Org. Chem.*, **27**, 3674 (1962).

matic compounds[6,7] has been accomplished with this reagent. Hydroxylation of the tetracycline D ring with this reagent seemed to be plausible. In initial experiments using the original conditions prescribed by Udenfriend, *et al.*,[6] only epimers and extensively oxidized residues were obtained. However, when the pH value of the reaction mixtures was adjusted to 3.0 and the gaseous oxygen was replaced by hydrogen peroxide, one or more biologically active products were detected in the oxidation mixtures. A number of tetracycline derivatives yielded this type of result. In general, these products displayed lower R_f values as compared with their parent compounds by paper chromatography in a 1-butanol–phosphate buffer (pH 3.0) system.

The major, biologically active products in the reaction mixtures derived from the oxidation of 6-demethylchlortetracycline, chlortetracycline, and 6-demethyl-6-deoxytetracycline were found to be the corresponding 9-hydroxy derivatives. The general methods of oxidation, isolation, and characterization of these products are similar. As a specific example, 6-demethylchlortetracycline was first oxidized by mixing 0.4 ml of 30% hydrogen peroxide with 100 ml of Udenfriend reagent[6] containing 100 mg of the tetracycline. The initial pH of the mixture was adjusted to 3.0 and the reaction mixture was kept at 23–25° for 45 min, freeze dried, and stored under a nitrogen atmosphere. Partial purification of this product was effected by the use of a partition column chromatographic system consisting of 1-butanol–chloroform–0.01 *N* hydrochloric acid (8:2:5). The desired yellow bands eluting at 1.3–3.0 hold-back volumes (HBV) were collected, the volume was reduced tenfold under vacuum, and the products were precipitated with *n*-heptane. The precipitates were washed, dried under vacuum, and rechromatographed in another partition system containing ethyl acetate–*n*-heptane–methanol–water (60:40:15:6). It was essential to adjust the pH of the sample solution to 5.6 to 6.0 immediately prior to loading the column. The fraction collected between 2.3 and 14.3 HBV was evaporated slowly under vacuum at a temperature below 10°, and a crystalline material began to precipitate.

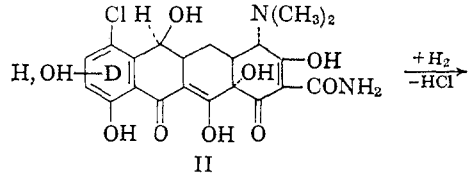
The material, after recrystallization, exhibited the ultraviolet and infrared absorption spectra patterns of a typical tetracycline antibiotic. The empirical formula was $C_{21}H_{21}N_2ClO_9 \cdot H_2O$. *Anal.* Calcd: C, 50.50; H, 4.63; N, 5.64; Cl, 7.04; O, 32.20. Found: C, 50.14; H, 4.98; N, 5.50; Cl, 6.98; O, 32.42. This suggested that one O had been introduced into the parent tetracycline. Comparison of the nmr spectra of the parent 6-demethylchlortetracycline (I) and the product (II) indicated the typical A/B quartet (469, 430 cps, J = 9 cps) in the aromatic region of I was a singlet (433 cps) in the spectrum of II. Thus it was evident that during the course of the reaction one of the aromatic hydrogens of I had been replaced by the new oxygen atom in the form of a hydroxyl.

When this hydroxy derivative of 6-demethylchlortetracycline was subjected to dechlorination with hydrogen and 5% palladium on alumina in triethylamine



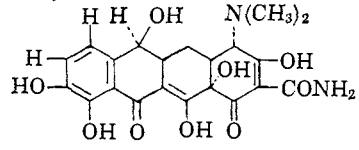
I

7-chloro-6-demethyltetracycline



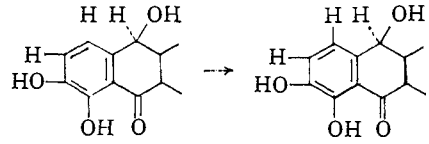
II

7-chloro-6-demethylhydroxytetracycline



III

6-demethyl-9-hydroxytetracycline



solution, a new hydroxylated 6-demethylhydroxytetracycline derivative was obtained.

The nmr spectrum of this product exhibited an A/B quartet (412, 432 cps) in place of the single peak shown by II. The coupling constant (J = 9.0 cps) is that associated with *ortho*-situated aromatic hydrogens. This indicates that the hydrogen atom adjacent to the chlorine atom of the parent tetracycline (I) was unaffected and the oxygen atom was introduced into C_9 (III) rather than C_8, for otherwise the product would have contained two *meta*-oriented hydrogen atoms.

These 9-hydroxytetracyclines exhibited both *in vivo* and *in vitro* activities against *Staphylococcus aureus*. The relative activities of some of these derivatives as compared with tetracycline are listed in Table I. In general, these new substances are less active than their parent compounds.

Table I. Microbiological Activities of Some 9-Hydroxytetracyclines against *S. aureus*

	Activity index
Tetracycline	1.0
9-Hydroxychlortetracycline	1.2
6-Demethyl-9-hydroxychlortetracycline	0.7
6-Demethyl-6-deoxy-9-hydroxychlortetracycline	1.2
6-Demethyl-9-hydroxytetracycline	0.2

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(6) S. Udenfriend, C. T. Clark, J. Axelrod, and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).
(7) C. E. Dalgliesh, *Arch. Biochem. Biophys.*, **58**, 214 (1955).