Noticeable differences in the chemical shifts of the 19-methyl protons for α- and β-epoxides were also anticipated.[8]

Eight $5\alpha,6\alpha$-epoxides and seven $5\beta,6\beta$-epoxides, all having either an ethylene ketal or a 3β-acetoxy substituent at C-3, were examined.[13,14] In no compound were there C═C or C═O functions which could give rise to long-range shielding of either the 19-protons or the epoxidic proton. For the α-epoxide-3-ketals the 6β-proton resonance always appeared within the range 169.5–171.5 c./s. from the TMS reference as a doublet, J, 3.3–4.1 c./s., while the 6α-proton resonance of the β-epoxide-3-ketals appeared at 183.0–185.4 c./s. from TMS,[15] J, 2.1–2.7 c./s. Replacement of the 3-ketal by 3β-OAc raises the epoxidic proton absorption range by *ca.* 5 c./s. for the α-epoxides and by *ca.* 2 c./s. for the β-epoxides. A comparison of observed and calculated J values is given in Table I. It is of note that these epoxides constitute very rigid systems, yet the observed J values for the epoxidic protons are *lower* than either set of calculated values. The agreement of the observed values and those calculated from the Karplus equations[5] is however acceptable. The range of J values is conveniently narrow in each case and coupled with the value of the chemical shift *completely defines the epoxide stereochemistry.* That each proton absorption appears as a doublet agrees well with the implications[5,7] that for ϕ = 70–110°, J is quite small. In both epoxides only *one* 6,7-proton coupling is observed.

TABLE I.

CALCULATED AND OBSERVED COUPLING CONSTANTS FOR 6-PROTONS OF STEROID 5,6-EPOXIDES[13,15]

$5\alpha,6\alpha$-epoxide	ϕ^a	J^6, c/s	J^7, c/s	Observed J, c/s
6βH–7αH	94 ± 4°	0.28– 0.1	0.0–0.27	.....
6βH–7βH	28 ± 4°	5.8 – 6.8	7.2–8.3	3.3–4.1
$5\beta,6\beta$-epoxide				
6αH–7αH	75 ± 4°	0.03–0.62	0.36–1.06	.....
6αH–7βH	49 ± 4°	2.8 –4.0	3.6 –5.0	2.1–2.7

[a] These values of ϕ are measured from models. Twelve measurements were made in each case and the majority fell well within the limiting range of ±4°.

From the positions of the 19-proton resonances it was possible to calculate additivity values[9,10,17] of + 15.0 c./s. for the α-epoxide, + 2.5 c./s. for the β-epoxide, and + 2 c./s. for the 3-ketal of the β-epoxide.[18] Thus, after epoxidation of a series of Δ^5-steroid 3-ethylene ketals the 19-protons resonated at 64.4–65.6 c./s. for five α-epoxides and at 60.2–60.7 c./s. for four β-epoxides. Agreement of calculated and observed 19-proton frequencies is a further criterion for the epoxide stereochemistry. Moreover, if the epoxide bears a 3β-hydroxy or 3β-acyloxy function, the 3α-proton is shifted 25–30 c./s. away from the TMS frequency in the α-epoxides only.[19]

We consider it important to note that additivity values for steroid methyl proton frequency shifts are applicable *only when the introduction of further substituents into the steroid nucleus does not cause serious alterations of the relative positions and orientations of the methyls and substituents capable of long-range shielding.* This is particularly important for unsaturated and keto steroids. Thus, additivity generally holds for steroids containing a $\Delta^{9(11)}$ double bond or a $5\beta,6\beta$-epoxide, but when both groups are present this is no longer true since now ring B is forced into a half-boat form and the position of the 19-methyl relative to both groups is changed. For example, $5\beta,6\beta$-epoxy-$\Delta^{9(11)}$-pregnene-3,20-dione-17α,21-diol diacetate 3-ethylene ketal has calculated and observed 19-proton resonances of 70.0 and 73.7 c./s. respectively, a disparity considerably larger than the agreement normally observable.[9,10,11]

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[8] The chemical shift of the methyl protons at C_{19} or C_{18} in steroids is determined by the overall shielding experienced, which is a net effect of the position and orientation of all neighboring single and double bonds, plus long-range shielding effects due to magnetic anisotropy of more distant groups of electrons. *Changes in stereochemistry can lead to considerable changes in long-range shielding* effects.[7,9,10] Failure to consider fully these effects has led to errors in interpretation in recently published work.[10,11] A more detailed discussion of some new aspects will appear shortly.[12]

[9] A. D. Cross and P. W. Landis, unpublished results.

[10] R. F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961).

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[13] N.m.r. spectra were obtained for purified chloroform solutions at 60 Mc. using tetramethylsilane (TMS) as an internal reference. A Varian A-60 spectrometer was employed, but final calibration is against spectra run on a Varian HR-60 instrument, suitably equipped for calibration by the standard side-band technique. Accuracy limits are of the order of ±1 c./s. for chemical shifts and ±0.3 c./s. for J values.

[14] The α-epoxides were: $5\alpha,6\alpha$-epoxy-androstan-$3\beta,17\beta$-diol diacetate, $5\alpha,6\alpha$-epoxy-androstan-17-one-3β-ol acetate, $5\alpha,6\alpha$-epoxy-androstan-3-one-17β-ol-17-acetate 3-ethylene ketal, $5\alpha,6\alpha$-epoxy-pregnan-3,20-dione 3,20-diethylene ketal, $5\alpha,6\alpha$-epoxy-pregnan-3,20-dione-21-ol 3,20-diethylene ketal, $5\alpha,6\alpha$-epoxy-pregnan-3,20-dione 3-ethylene ketal, $5\alpha,6\alpha$-epoxy-pregnan-3,20-dione-21-ol 3-ethylene ketal 21-acetate, and $5\alpha,6\alpha$-epoxy-pregnan-3,20-dione-17α,21-diol 3-ethylene ketal 17α,21-diacetate. The β-epoxides were: $5\beta,6\beta$-epoxy-pregnan-3,20-dione-21-ol 3-ethylene ketal 21-acetate, $5\beta,6\beta$-epoxy-pregnan-3,20-dione 3,20-diethylene ketal, $5\beta,6\beta$-epoxy-pregnan-3,20-dione-17α,21-diol 3,20-diethylene ketal 17α,21-diacetate, $5\beta,6\beta$-epoxy-androstan-17-one-3β-ol acetate, $5\beta,6\beta$-epoxy-17α-ethinylandrostan-$3\beta,17\beta$-diol 3β-acetate, $5\beta,6\beta$-epoxy-androstan-$3\beta,17\beta$-diol diacetate, and $5\beta,6\beta$-epoxy-androstan-3-one-17β-ol 17-acetate 3-ethylene ketal.

[15] On the τ scale[16] the α-epoxide 6β-proton is *ca.* 7.2 and the β-epoxide 6α-proton is *ca.* 6.9.

[16] G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

[17] J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).

[18] Positive shifts are *away* from TMS, for the 19-proton resonance frequency, due to the extra deshielding induced by these functional groups, relative to 5α- or 5β-androstane, according to stereochemistry at C_5.

[19] Shifts of the 3α-H resonance have been noted for the other steroids with a highly polar 5α bond.[9]

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THE ANODIC OXIDATION OF TRIPHENYLMETHANE DYES

Sir:

We wish to report an unusual electrochemical reaction, which is exhibited in the anodic oxidation of crystal violet and related triphenylmethane dyes.

Crystal Violet (CV) and Malachite Green (MG), as well as *p,p'*-methylenebis-(N,N-dimethylaniline) (MBis) all yield N,N,N',N'-tetramethyl-benzidine (TMB) and its corresponding diquinoid (TMBOx) upon oxidation at platinum and carbon electrodes in acidic, aqueous buffers. It can be shown that this reaction must take place *via* ejection of an integral unit composed of the central carbon atom attached to a phenyl group. In the case of the MBis, where the central group is merely $—CH_2—$, formaldehyde results. The ethylated dyes Ethyl Violet (EV) and Brilliant Green (BG) which are analogous to Crystal Violet and Malachite Green, respectively, yield the corresponding N,N,N',N'-tetraethylbenzidine. These results are especially interesting in view of the recent work of Eastman, Engelsma and Calvin, who showed CV was formed by oxidation of dimethylaniline with chloranil.[1] Here the central carbon of the CV cation must originate from a methyl carbon of dimethylaniline. Our results show the central carbon residue can be removed by the relatively mild process of electrochemical oxidation. Thus it would appear that the central carbon of triphenylmethane dyes (and related compounds) is an unusually facile portion of a rather complex molecule. These results appear to be of fundamental interest in complex organic oxidation–reduction processes.

The cyclic voltammetry and other electrochemical techniques used here were identical with those reported in the study of the anodic oxidation of N,N-dimethylaniline.[2]

In 1 *N* sulfuric acid–sodium sulfate medium, CV oxidizes at *ca.* + 0.8 v. *vs.* s.c.e. Using a 2 v./min. triangular wave sweep voltage, no evidence of any oxidation at less than + 0.8 v. is evident on the *first* anodic sweep. However, on the second and all subsequent sweeps, an almost reversible oxidation-reduction system is found at the lesser anodic potential of *ca.* + 0.55 v. The anodic and cathodic half-peak potentials of this system correspond *within 2 millivolts* with those of TMB-TMBOx in the same medium.

Chemical oxidation of triphenylmethane dyes to TMBOx is fairly well established.[3,4] The most recent work of Hanousek and Matrka on MG is probably most definitive.[5] To have further proof that the compound formed electrochemically was TMBOx, CV, MG and MBis were oxidized with lead peroxide in sulfuric acid and the corresponding oxidation products were isolated as perchlorates. Solutions of all these compounds showed cyclic voltammetry in complete agreement with the TMB-TMBOx oxidation-reduction system formed by electro-chemical means only.

Further, these oxidation products were dissolved in 50% acetone–50% aqueous buffer of pH 3.8. These solutions all showed electron paramagnetic resonance (e.p.r.) spectra identical with that of the cation radical of TMB. This spectrum recently has been interpreted in detail.[6]

Finally, the uncertainty exists that, (a) one molecule of TMB arises by oxidation of 2 molecules of dye each losing a N-substituted phenyl, followed by chemical coupling reactions, or, (b) that one dye molecule loses the central carbon phenyl unit and the remaining two N-substituted phenyl groups couple *intra* to give TMB. It can be shown that the latter is the case. The peak current due to TMB which arises from MG, CV or MBis is *ca.* 1.8 times that obtained from similar oxidation of dimethylaniline. Since it has been shown that 2 moles of dimethylaniline are oxidized to give 1 mole of TMB, these results can be interpreted to mean that 1 mole of MG, CV or MBis gives 1 mole of TMB.

Another interesting facet of the triphenylmethane dye oxidation is that it occurs in a 2-step wave. It can be shown that the two waves are due to the oxidation of the hydrated and non-hydrated forms of the dye proposed by Cigen.[7] It is only the hydrated form which gives rise to the TMB during anodic oxidation in strong acid medium. A detailed interpretation of the electrochemical results will be given soon.

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ON THE MECHANISM OF THE ENZYMATIC DECARBOXYLATION OF ACETOACETATE. II[1]

Sir:

The decarboxylation of acetoacetate by the decarboxylase[2] purified from *Cl. acetobutylicum* previously had been shown to involve obligatory exchange of the carbonyl oxygen atom with the oxygen of the water used as solvent.[3] These findings suggest[3] that a Schiff base formed between the enzyme and its substrate may be an active intermediate in the decarboxylation. We have therefore tried to trap the postulated Schiff base intermediate by reduction with borohydride as has been done with the Schiff bases present in other enzymic[4–6] and similar systems.[7] The successful results of these experiments are reported below.

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