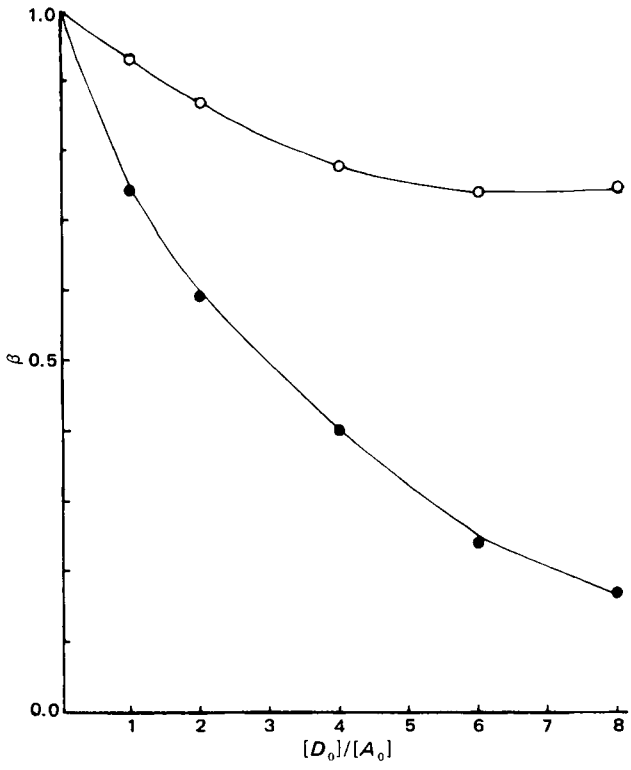

Figure 3—*Change in the relative fluorescence intensity of (−)-tryptophan, β, versus the molar ratio of catechol to (−)-tryptophan, $[D_0]/[A_0]$, at 10^{-5} M (-O-O) and 10^{-4} M (-●-●) (−)-tryptophan in cacodylate buffer (10^{-3} M) at pH 7 and 15°. The excitation wavelength was 275 nm. Fluorescence intensity was measured at 365 nm.*

to eightfold) were used. Furthermore, catechol did not have appreciable fluorescence at the highest concentration used (~5% from the total fluorescence intensity at the wavelength used).

In correlating the equilibrium constants obtained by the methods, a quenching coefficient, γ, is introduced in calculating the true (thermodynamic) equilibrium constant, K_t, from the simple mathematical model proposed:

$$K_{fluo} = \frac{[AD]\gamma_{AD}}{[A]\gamma_A[D]\gamma_D} = \frac{[AD]}{[A][D]}\left(\frac{\gamma_{AD}}{\gamma_A\gamma_D}\right) = K_t K_{quen} \qquad \text{(Eq. 3)}$$

$$K_{quen} = \frac{\gamma_{AD}}{\gamma_A\gamma_D} \qquad \text{(Eq. 4)}$$

where K_{quen} is the quenching constant. When K_{quen} was calculated by assuming $K_t \simeq K_{abs}$, it had a value of 3.4562×10^4.

Fluorescence quenching of tryptophan and some other fluorescing compounds was attributed to charge transfer interactions (4). In those studies, tryptophan was considered as an electron donor. It was suggested that the catechol-containing compounds act as electron donors when forming charge transfer complexes with nucleic acid bases in aqueous acidic solutions (1, 2). It may be reasonably concluded that the results presented in this work can be interpreted on the basis of charge transfer effects. It is difficult to assign the donor or acceptor species of the present molecules since precise theoretical data involving molecular orbital calculations of the highest occupied or lowest empty molecular orbitals are either inconsistent or unavailable and the values usually are expressed in terms of resonance units (uncertain quantities). However, it is assumed tentatively that catechol acts as an electron donor while (−)-tryptophan acts as an electron acceptor.

To explain the differences between the equilibrium constants obtained from the two methods as well as the appearance of the quenching constant, it is proposed that one pair of (−)-tryptophan–catechol out of several thousand pairs of molecules, within the concentration limits used, exists in close proximity in the form of a real complex while the rest remain isolated in solution.

On the other hand, to justify the charge transfer mechanism for the observed quenching, we propose a hypothesis of an electron pump through water. Experimental results indicated an observable quenching at concentrations as low as 10^{-5} *M* each (5.5%). Simple calculations revealed that the distance between molecules at 2×10^{-5} *M* is 436.26 Å; the distance between two molecules of water is 3.1 Å. Therefore, under this proposal, an electron can be pumped through ~140 molecules of water.

It is hoped that the results presented here and the theory advanced may help in understanding many important biological processes such as conduction through nerves and other excitable membranes.

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Hanna N. Borazan ˣ
Yousif H. Ajeena
Department of Pharmaceutical Chemistry
College of Pharmacy
University of Baghdad
Baghdad, Republic of Iraq

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Mass Spectra of Dexamethasone and Betamethasone

Keyphrases □ Dexamethasone—mass spectral analysis, comparison with mass spectrum of betamethasone □ Betamethasone—mass spectral analysis, comparison with mass spectrum of dexamethasone □ Mass spectrometry—comparison of spectra of betamethasone and dexamethasone

To the Editor:

The application of mass spectrometry to the study of steroids provides a powerful tool for establishing the structure and spatial configuration of these complex molecules (1). In particular, the decomposition of steroidal alcohols under electron impact often is specific, with the position of the hydroxyl group affecting the fragmentation pattern of 17-hydroxysteroids, thus allowing discrimination between epimeric alcohols and their derivatives.

However, the occurrence of unwanted phenomena, *e.g.*,

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Table I—Relative Abundances of the Peaks at *m/e* 315 and 343 in the Mass Spectra of Betamethasone and Dexamethasone

Compound	*m/e*	Relative Abundance[a], %
I	315	3 ± 2[b], 3, ~10
	343	3 ± 2[b], 5, ~12
II	315	40 ± 5[b], 12, ~15
	343	4 ± 2[b], 5, ~76

[a] Refers to the base peak at *m/e* 122. The first value was determined by the present investigator, the second value was taken from Ref. 5, and the third value was obtained from Ref. 2.

thermal decomposition of the sample under the rather drastic conditions prevailing in the ion source, must be given serious consideration with steroids to prevent experimental artifacts.

A case in point is the mass spectral analysis of betamethasone (I) and dexamethasone (II), which are configurational isomers at C-16; their spectra were reported by Lodge and Toft (2, 3). According to these investigators, unequivocal discrimination between the epimers is allowed by the fragment (M − 49) at *m/e* 343, a major peak (actually, the second most abundant ion) in the spectrum of II whose abundance is insignificant in the spectrum of I. Formation of the fragment was traced (2, 3) to the preliminary loss of water from the D-ring followed by cleavage of the 20,21-bond, which should be favored in II according to a mechanism similar to that suggested by Zaretskii *et al.* (4) for the epimers of 17-alkyl-19-nortestosterone.

While these results and their interpretation remain unchallenged in the literature, there are indications (5) of unsuccessful attempts to corroborate the occurrence of a major peak at *m/e* 343 in the mass spectrum of II together with the suggestion that the spectrum may be affected greatly by the thermal decomposition of the sample. In view of the pharmaceutical relevance of the epimeric pair involved, a systematic investigation was undertaken to reconcile the discrepancy and to evaluate the effects of the possible instrumental artifacts.

Authentic samples of I and II from different sources, whose identity and purity had been checked by melting-point determination, IR spectroscopy, and high-pressure liquid chromatography (HPLC), were analyzed on a quadrupole spectrometer[1] and two magnetic sector spectrometers[2]. An effort was made to duplicate as closely as possible the conditions described previously (2, 3), using a direct-insertion probe temperature of 180–200° and an electron energy of 70 ev.

The results confirm that the molecular ion is barely detectable and indicate that the base peak in the spectra of both I and II is the ion at *m/e* 122. However, other significant features of the spectra, including the peaks at *m/e* 315 and 343, differ significantly from previously reported values (Table I).

The discrepancies clearly are too large to be explained by instrumental factors and suggest that thermal decomposition of the sample plays a significant role. This view is supported by the correspondingly large scatter (~22°) of the melting points reported in the literature and their dependence on the heating rate (5).

To gather additional evidence on this point, further experiments were carried out using thermogravimetric analysis and mass spectrometry. A sample of II was heated in a thermoanalyzer[3], both in a self-induced atmosphere and in dry nitrogen, from 25 to 220° at rate of 4°/min and then maintained at 220°. A significant (8.1 ± 0.1%) weight loss occurred above 200°, peaking at ~205°.

In a separate experiment, samples of II were heated at 205° in a glass tube under high vacuum (1.5×10^{-6} torr), and a white sublimate was observed immediately above the heated portion of the tube. The composition of the sublimate could be taken as representative of the composition of the vapors above the heated sample under conditions that closely approach those in the ion source. Accordingly, the mass spectrum of the sublimate was recorded and was significantly different from that of pure II. In particular, the relative abundances of the peaks at *m/e* 315 and 343 were 13 and 7%, respectively, which are very close to the values reported in Ref. 5.

These experiments provide compelling evidence that thermal decomposition plays a significant role in the mass spectrum of II, affecting in particular the relative abundances of the peaks at *m/e* 315 and 343. Consequently, the diagnostic value of the fragment at *m/e* 343 in unequivocally distinguishing between I and II is questionable.

If discrimination between the two epimers has to be achieved by mass spectrometry rather than by more suitable techniques, *e.g.*, HPLC, it appears that the most significant diagnostic peak is the one at *m/e* 315. In fact, not only the present data but also those reported in Ref. 5 show that the fragment at *m/e* 315 is consistently and significantly more abundant in the mass spectrum of II than of I.

Any mechanistic interpretation of the electron-induced fragmentation pattern of complicated molecules, especially in those cases where heat-sensitive substances are involved, should be made cautiously.

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Marina Attinà
Institute of Pharmaceutical Chemistry
University of Rome
00100 Rome, Italy

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[1] Hewlett-Packard model 5980.
[2] AEI model MS-12 and Varian model MAT 311A.
[3] Model TA1, Mettler Co.