Unequivocal Distinction between Betamethasone and Dexamethasone by Mass Spectrometry

Keyphrases ☐ Dexamethasone, betamethasone—distinction, determination ☐ Mass spectrometry—identity

Sir:

In a review on stereoisomeric effects on mass spectra, Meyerson and Weitkamp (1) state that mass spectra of stereoisomers are, in general, qualitatively and quantitatively similar. Reference is made therein to work that demonstrates differences between the mass spectra of epimeric pairs of secondary and tertiary steroid alcohols. More recently, Grostic and Rinehart (2) have distinguished between epimeric 11-hydroxyprogesterones by mass spectrometry.

We wish to report that mass spectrometry provides an unequivocal method of distinguishing between the epimeric pair of synthetic corticosteroids betamethasone (9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-dien-3,20-dione) and dexamethasone (9α-fluoro-11β, 17α,21-trihydroxy-16α-methylpregna-1,4-dien-3,20-dione). The difference is large enough to be of considerable value in forensic analysis. The spectra, which will be discussed fully at a later date in combination with spectra of related compounds, are shown (Fig. 1 = betamethasone, Fig. 2 = dexamethasone). The principal distinguishing feature is the peak at *m/e* 343, which corresponds to a loss of 49 mass units. In the dexamethasone spectrum, this peak is the next most prominent after the base peak (*m/e* 122); whereas in the spectrum of betamethasone, it is relatively insignificant.[1]

The peak at *m/e* 343 (M − 49) almost certainly arises from loss of water from the D-ring followed by cleavage of the 20,21-bond. Dexamethasone has a *trans*-diaxial arrangement of the 16β-hydrogen and 17α-hydroxyl, which favors dehydration. In betamethasone, there is no hydrogen *trans*-diaxially oriented to the 17α-hydroxyl. An analogous situation has been reported by Zaretskii *et al.* (3).

The high sensitivity of mass spectrometers means that this distinction may be carried out with a very limited amount of material, particularly in view of the relative abundance of the M − 49 peak in the spectrum of dexamethasone. There is, in addition, the advantage

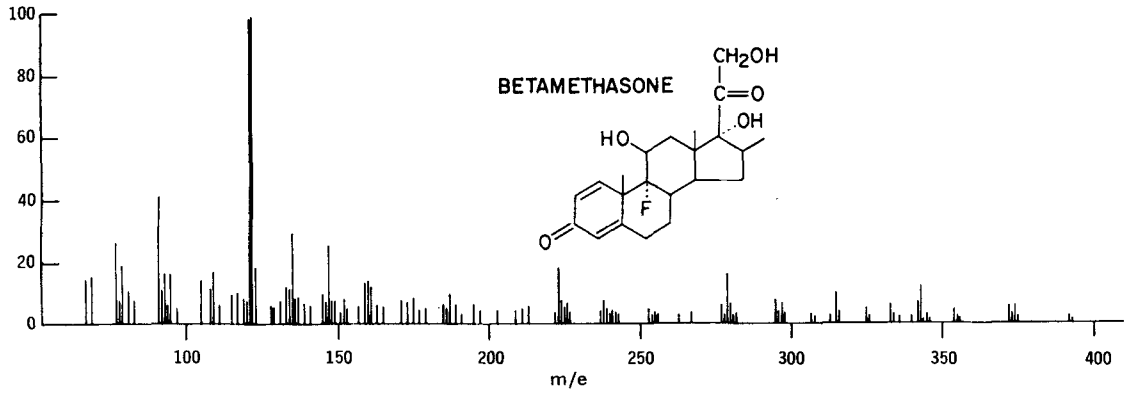

Figure 1—*Mass spectrum of betamethasone.*

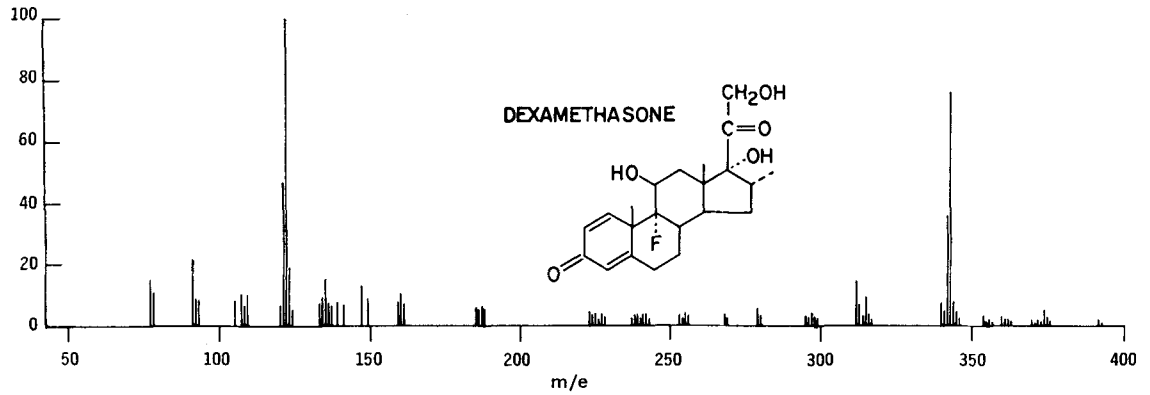

Figure 2—*Mass spectrum of dexamethasone.*

[1] Mass spectra were recorded on a Hitachi-Perkin-Elmer model RMU-6D with an ionization voltage of 70 ev. The compounds were introduced directly into the ion chamber.

for analytical purposes that the molecular weight of the substance is indicated.

(1) S. Meyerson and A. W. Weitkamp, *Org. Mass Spectrom.*, **1**, 659(1968).

(2) M. F. Grostic and K. L. Rinehart, *J. Org. Chem.*, **33**, 1740 (1968).

(3) V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, V. N. Leonov, and I. V. Torgov, *Tetrahedron*, **24**, 2339(1968).

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Audiosensitization: Potential Screening Method for Drugs Affecting the CNS

Keyphrases □ CNS active drugs—screening method □ Stress, sound induced—seizures

Sir:

With the advent of new types of CNS active drugs, new screening tests with predictive association for subtle drug effects are needed. The phenomenon of audioconditioned convulsions (1, 2) affords unique potential as such a screen.

The exaggerated and abnormal responses of psychiatric patients to auditory stimuli (3) prompted the use of audiogenic seizures in genetically susceptible strains of mice as an analogous reaction pattern for CNS drug research (4, 5). The present report suggests the use of audioconditioning and the subsequent susceptibility to sound-induced seizures as a simple and more informative analogy. Both analogies are based on the hypothesis that stress-induced neurosis can be measured by quantal observations of CNS hyperexcitability in response to a specific triggering mechanism. The use of audioconditioning has the advantage of offering the induction of stress susceptibility, as well as the stress-induced crisis for drug modification and study.

Susceptibility to sound-induced seizures can be conditioned in "sound-resistant" strains of mice by a short period (30 sec.) of auditory stimulation at a critical early age (1, 2). In mice, early experiments with the classical conditioning method of physiology showed that pretest exposure to sound elevates or reduces seizure threshold, depending upon the temporal parameters of treatment (6). In such reports, however, the durations of both the conditioning stimulus and the condition–test interval have been short, generally only a few seconds. The audioconditioned convulsions described here are inherently similar, but the condition–test interval is much longer and is measured in days.

Sound-resistant mice [*e.g.*, CAW:CF-1 (SW)][1] display an auricular startle upon initial sound exposure (audioconditioning), but less than 5% convulse (2). The sound source is a 6.3-cm. doorbell which produces approximately 95 db. (relative to 0.0002 dyne/cm.^{2}) within a glass testing chamber, 25 cm. in diameter by 15 cm. deep. If conditioned at the optimally sensitive age (18–20 days for CF-1), virtually all mice will convulse upon the second (test) sound exposure 2–3 days later. The initial conditioning stimulation is absolutely essential for the genesis of convulsions.

The typical seizure in such sensitized animals consists of a sudden burst of wild running, followed by clonic and then tonic convulsions. Less severe seizures terminate after running or clonus. Estimates of seizure severity can be derived from latency and duration times as well as from seizure pattern (2, 7). The following experimental factors affect these parameters (2, 7, 8):

1. The interval between conditioning and testing is critical. Maximal clonic–tonic convulsions characterize seizures after a 2- or 3-day condition–test interval; with a 1- or 5-day interval, only clonus is seen.

2. Repeated auditory stimulation prior to the development of convulsibility makes mice temporarily refractory to seizure, and prolongation of the initial conditioning sound (over 6 hr.) imparts permanent seizure resistance without causing deafness. Once an animal experiences a convulsion, however, seizure susceptibility persists for several weeks. This indicates that audiosensitization and seizure susceptibility are mediated by separate mechanisms.

3. The tonal characteristics of the sound stimulus are equally or more important than the intensity. Although reproducibility is excellent, it is necessary to bioassay each bell periodically. After extensive use, a bell may no longer induce maximal seizures, despite no alteration in intensity.

4. Genetic and environmental factors must be controlled. Noises in the animal quarters, such as the clatter of metal garbage cans, have profound influence. The critical age for sensitization and the optimum condition–test interval vary from strain to strain. CF-1 mice have a high incidence of maximal seizures, a short duration of audiosensitivity, and a low death risk.

When these experimental factors are controlled, seizures of predictable incidence, severity, and latencies are produced (2).

Theoretically, pharmacologic alteration of audioconditioned seizures should be afforded by: (*a*) drugs that impair hearing or otherwise interfere with input of the sound stimulus; (*b*) drugs that block central perception of the stimulus; (*c*) drugs that inhibit or enhance the slow process of sensitivity development; (*d*) drugs that block the effect of intertrial stress; and (*e*) drugs that modify the mechanism of seizure production. The novel interest in audioconditioning as a screen will be for drugs that alter the development of sensitization (*b* and *c* as previously mentioned). For these drugs, this screen is unique because the potential drug need not be present at the time the animals are challenged for a test response. Thus, the prosensitizing

[1] Carworth Farms, New City, N. Y.