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## Metabolic Pattern of Mibolerone

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### Introduction

Mibolerone (17 $\beta$ -hydroxy-7 $\alpha$ ,17-dimethylestr-4-en-3-one; Cheque; Matenon) binds most tightly to the steroid receptor of any synthetic steroid. It is primarily used to prevent estrous in dogs and cats. It has not been approved for use in man due to its high potential for hepatotoxicity. It has been preliminarily identified by the United States Drug Enforcement Agency in confiscated drugs.

There are few metabolic studies available on mibolerone. Studies with radioactive compounds in chickens and dogs located 5-10 metabolites with chronic administration [1,2]. The structural identity of these metabolites was not reported. Some "oxidative products" have been observed in aqueous solution[3].

### Experimental

Urine samples were extracted and derivatized according to the standard conjugated steroid procedure. The steroids were separated on a 0.25 mm x 15 m, 25  $\mu$ m film thickness DB-1 capillary GC column.

### Results and Discussion

Mibolerone standard eluted very close to the internal standard 17-methyltestosterone, with a relative retention time of 0.97. The major ions observed were m/z 431 (loss of methyl; base peak); 446 (molecular ion; 85% relative abundance); 301 (85% relative abundance); 341 (loss of TMS+methyl; 40% relative abundance); and 356 (loss of TMS; 20% relative abundance).

Bolasterone has a relative retention time in this system of 1.01.

The tetrahydro-mibolerone metabolite had a relative retention time of 0.88. The major fragment ions were m/z 360 (loss of TMS; 10% relative abundance); 270 (loss of two TMS; 25% relative abundance); and a base peak of 143. Very little of the molecular ion (m/z 432; 1% relative

abundance) was observed. For reference purposes, the relative retention time of tetrahydrobolasterone in this system is 0.94.

Mibolerone was essentially undetectable after 24 hours. The metabolite concentration peaked at 48-60 hours, and could be detected for five days.

### Conclusions

Mibolerone and its reduced metabolites are present in the glucuronide fraction. Based on the low resolution mass spectrum, the major metabolite is proposed to be a tetrahydro compound, most likely estra-7 $\alpha$ ,17 $\alpha$ -dimethyl-3,17-diol. Peak concentrations occur at about 36 hours. The metabolite is detectable for five days after a single 1 mg dose.

### References

1. G.H. DUNN, L.F. KRZEMINSKI, R.E. GOSLINE, AND C.J. SUBACZ: Metabolism of [<sup>14</sup>C]Mibolerone in the chicken. *J. Agric. Food Chem.* 27 (1979) 1001.
2. L.F. KRZEMINSKI, J.H. SOKOLOWSKI, G.H. DUNN, F. VANRAVENSWAAY, AND M. PINEDA: Serum concentrations of mibolerone in beagle bitches as influenced by time, dosage form, and geographic location. *Am. J. Vet. Res.* 39 (1978), 567.
3. M.I. AMIN, K.T. KOSHY, AND J.T. BRYAN: Stability of aqueous solutions of mibolerone. *J. Pharmaceut. Sci.* 65 (1976), 1777.