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Effect of Sulfinpyrazone on Urinary Secretion of Steroid Conjugates

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Introduction

Pharmacological agents can alter renal clearance of drugs and endogenous compounds either by blocking organic anion transport from serum into renal tubular cells at the basolateral surface and secretion of the anions into the lumen or by blocking reabsorption of the organic anions from the kidney lumen. Probenecid is the classic agent for blocking secretion of organic anions, including steroids [1]. Sulfinpyrazone (Anturan) has also been reported in the literature as an "inhibitor of organic anion transport". It has been observed to increase the renal clearance of methotrexate [2] and 6β-hydroxycortisol [3], presumably by blocking tubular re-absorption of the compounds. Anomolous behavior has been observed for uricosuric agents, however, since transport inhibition is a function of drug concentration and competitive interactions with other drugs binding to the same receptor. We report here on studies to evaluate the effect of sulfinpyrazone on the urine concentration of both endogenous and exogenous conjugated steroids.

The pharmacokinetics of sulfinpyrazone have been well studied. Most of the dose (55%) is secreted in the urine unchanged [4,5]. About 10% of the ingested dose is reportedly present in urine as *p*-hydroxy-sulfinpyrazone, the main metabolite of sulfinpyrazone. An unusual C-linked glucuronic acid conjugate has also been reported [4]. Sulfinpyrazone and its metabolites have been monitored in both plasma and urine by HPLC and by GC [6].

Experimental

Two volunteers ingested a standard dose of 200 mg Anturan®, and one volunteer ingested 400 mg. One of the low dose volunteers also ingested a 20 mg dose of norethandrolone on two consecutive days; the first without sulfinpyrazone and the second day subsequent to the steroid dose.

Both pure sulfinpyrazone and urine samples after oral ingestion were analyzed for unconjugated basic drugs (Procedure I), conjugated basic drugs (Procedure II), and steroids (Procedure IV). The concentrations of androsterone, etiocholanolone, 11β-hydroxy-androsterone, 11β-hydroxyetiocholanolone, epitestosterone, and testosterone were determined from the standard

method for conjugated steroids. Briefly, this consists of separation of conjugates on a C-18 solid phase extraction column, hydrolysis with *H. pomatia* β-glucuronidase, extraction with methyl-*t*-butylether, and derivatization with MSTFA/TMSI/dithioerythritol. Methyltestosterone was used as the intenal standard. Analysis was carried out on a Hewlett Packard 5890/5970 GC/MSD. The concentration versus time profile of norethandrolone metabolites was followed on two days, one with and one without sulfinpyrazone dosage.

Results and Discussion

Sulfinpyrazone could be readily detected in both Procedure I and in the steroid screening procedure. The relative retention time in Procedure I was 1.64, using diphenylamine as the internal standard. The spectrum observed on GC/MS analysis of the underivatized sulfinpyrazone was matched by the library spectrum in the standard Hewlett Packard version of the Pfleger, Maurer, and Weber library. The GC/MS spectrum of the TMS derivative of sulfinpyrazone is shown in Figure 1.

No significant effects of sulfinpyrazone were observed on either the peak concentration or on the excretion rates of the endogenous steroids (Table 1).

Table 1. Relative concentrations of Endogenous Steroids 4-8 hours after Administration of Sulfinpyrazone to Three Volunteers

	% Concentration*					
Volunteer	**Androst**	**Etiochol**	**Testo**	**Epitesto**	**11β-Androst**	**11β-Etiochol**
200 mg Dose	100	113	109	92	96	107
200 mg Dose	110	114	122	102	99	106
400 mg Dose	119	105	152	107	113	97

* Calculated as a percentage of the pre-dose concentration

Table 2 shows the results of two sequential administrations of norethandrolone to a subject; the second administration was preceeded by two hours with a dose of sulfinpyrazone. Neither the excretion rates of the exogenous steroid metabolites nor any endogenous steroids were significantly altered by this uricosuric drug.

Table 2. Time course of relative excretion of Norethandrolone metabolites, Androsterone, and Testosterone with and without 200 mg Sulfinpyrazone Administration

	Norpregnanetriol (µg/mg creat)		Tetrahydro-norethandrolone		Androsterone (µg/mg creat)		Testosterone (µg/mg creat)	
Time Post Dose (h)	S−	S+	S−	S+	S−	S+	S−	S+
0-2	1.08		0.23		2.81		0.049	
2-4	3.33	2.33	1.29	1.22	2.18	2.16	0.042	0.039
4-8	4.56	4.10	1.30	1.29	2.05	2.15	0.030	0.031
8-12	5.73	3.99	5.73	5.50	1.50	1.17	0.018	0.017
12-24	2.83	1.12	0.42	0.29	1.97	1.31	0.032	0.028

Conclusions

Sulfinpyrazone does not effect the excretion of conjugated steroids at the doses tested. In fact, there was a slight, but statistically insignificant, increase in steroid concentration in the urine. This might be expected given the fact that sulfinpyrazone functions by inhibiting reabsorption of organic anions. It is possible that the anomolous behavior noted above could result in inhibition of secretion at lower doses. Although unlikely, this possibility is under investigation. Given its similar mechanisms of action, it is likely that benzbromarone is similarly ineffective at blocking steroid secretion, although no systematic studies of benzbromarone effects on anion transport have been reported. Nevertheless, the detection of sulfinpyrazone in urine might be construed as an attempt, albeit unsuccessful, at urine manipulation. Sulfinpyrazone is detectable in both the PI and conjugated steroid procedures.

References

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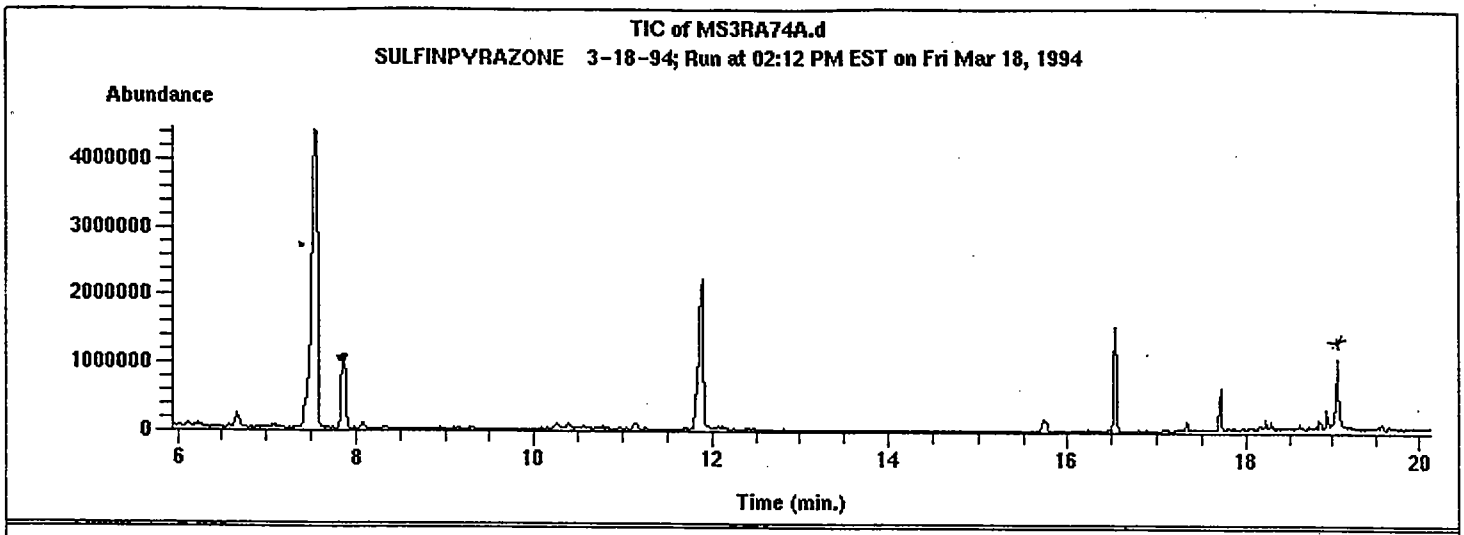
FIGURE CAPTIONS

Figure 1. Total ion current chromatogram from the conjugated fraction of procedure IV showing the retention of sulfinpyrazone (19.04 min) and methyltestosterone (16.55 min). The lower portion of the drawing shows the mass spectrum of the TMS derivative of sulfinpyrazone along with a proposed fragmentation pattern.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10
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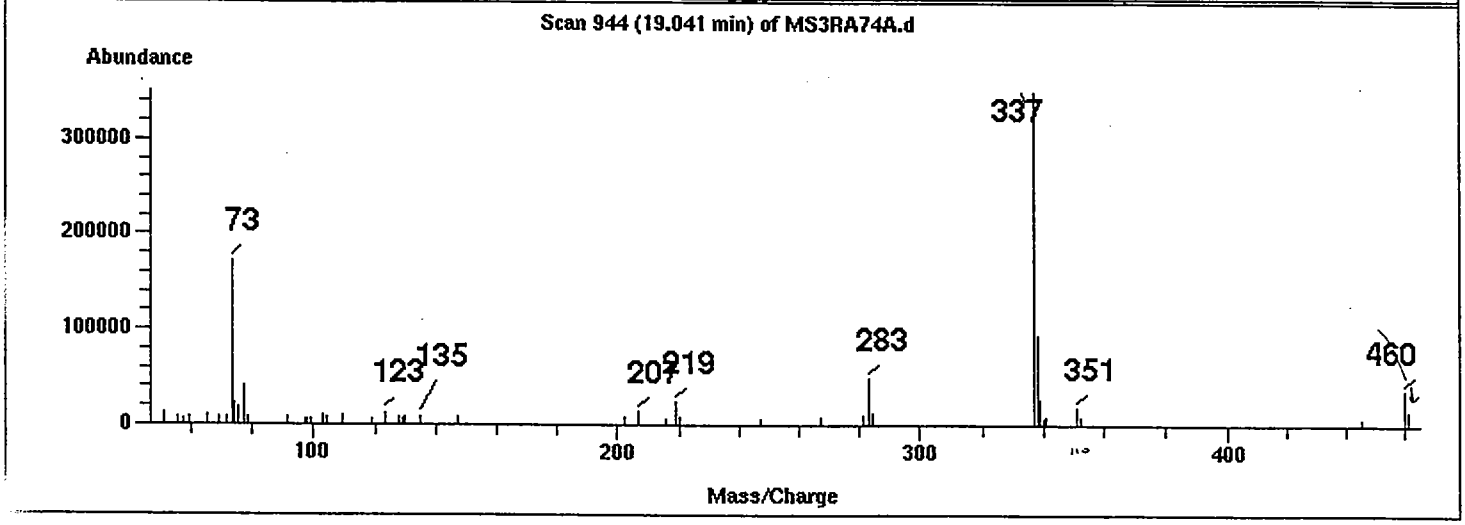
Time (min.)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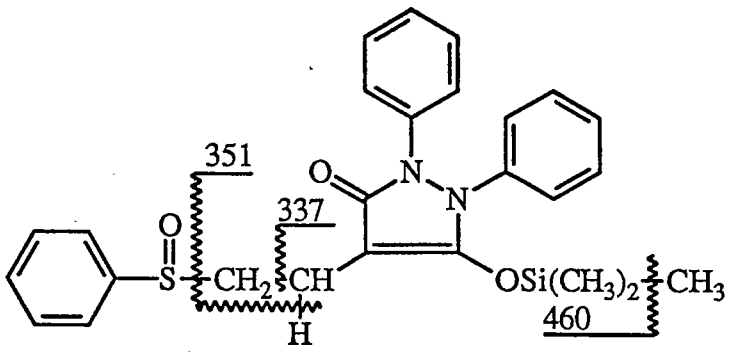
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337
460
O
N
N
O
S
CH2
CH
H
OSi(CH3)2
CH3