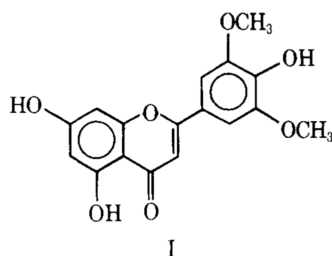

identical by mixed melting-point, IR, UV, and NMR comparisons with an authentic[4] sample of tricin (I) (4, 5).

The ^{13}C-NMR spectrum of tricin was taken in dimethyl sulfoxide-d_6 with tetramethylsilane as the internal standard, and the chemical shifts corresponding to the various carbon atoms in the molecule are given in Table I. The chemical shifts were assigned by comparison with the data published (6) for other flavonoid compounds. The shift for C-7 and C-2 can be interchanged. The relative lowfield shifts of C-10 and C-1′ carbons are probably due to the deshielding effect caused by the interaction of the solvent molecules dimethyl sulfoxide-d_6 with the hydroxyl functions *ortho* and *para* to C-10 and C-1′, respectively.

Table I—^{13}C-NMR Chemical Shift Assignments for Tricin

Carbon	Chemical Shift, ppm	Carbon	Chemical Shift, ppm
2	164.0	9	161.3
3	103.6	10	120.8
4	181.6	1′	139.7
5	157.2	2′, 6′	104.3
6	98.7	3′, 5′	148.0
7	163.5	4′	164.0
8	94.1	OCH_3	56.3

EXPERIMENTAL

Air-dried and ground tops of *S. cynosuroides* (10.0 kg) were extracted with 95% ethanol for 24 hr, and the extract was concentrated *in vacuo*. The gummy residue (Fraction A) was treated with 5% acetic acid (2000 ml) and stirred thoroughly. The acidic solution was then filtered through a bed of diatomaceous earth[5], and the filtrate was cooled in ice. Basification of the filtrate with ammonium hydroxide to pH 4 gave a precipitate, which was thoroughly extracted with chloroform. The organic layer was then washed free from acid, dried over anhydrous sodium sulfate, and distilled *in vacuo* to give a dark residue (Fraction B, 15 g).

Tricin—Fraction B was dissolved in chloroform (100 ml) with a few drops of methanol and then chromatographed in a column (50 × 2.5 cm) of silica gel G. Fractions of 500 ml each were eluted successively with chloroform (10 liters) and chloroform–ethanol (99:1, 10 liters). Fractions eluted with chloroform–ethanol (99:1, 5 liters), upon evaporation, gave a yellowish-brown residue (0.5 g). This residue was crystallized several times from chloroform–methanol to give a yellow powder, mp 288–290° dec.

Tricin Triacetate—Tricin (0.066 g, 1.02 mmoles) was treated with acetic anhydride (1 ml) and dry pyridine (1 ml) for 16 hr at room temperature. After the usual workup, the residue was crystallized from ethyl acetate as white needles (0.045 g), mp 263–265° dec.

[4] Kindly supplied by Dr. J. B. Harborne, Department of Botany, University of Reading, Reading, England.
[5] Celite.

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Sex Differences in Plasma Half-Life of Dextrorphan in Rats Administered Dextromethorphan

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Abstract □ Male and female rats exhibited striking differences in their ability to metabolize dextromethorphan. Upon oral administration of this drug, the plasma half-life of dextrorphan, a major biotransformation product formed by *O*-demethylation, was 40 min in the female rat and 18.5 min in the male.

Keyphrases □ Dextromethorphan—metabolism in male and female rats compared □ Dextrorphan—plasma half-life in male and female rats compared □ Metabolism—dextromethorphan in male and female rats compared □ Antitussives—dextromethorphan, metabolism in male and female rats compared

Investigations in these laboratories on the bioavailability of dextromethorphan hydrobromide, a nonnarcotic antitussive agent, revealed striking differences between male and female rats in their metabolism rates. Upon oral administration, it is rapidly metabolized, the main routes of biotransformation being *O*- and *N*-demethylation, followed by conjugation of the desmethyl metabolites to glucuronides and sulfates (1–5).

Determination of the plasma levels of dextrorphan (*d*-3-hydroxy-*N*-methylmorphinan), the *O*-demethylated metabolite, previously (6) was employed to evaluate the bioavailability of dextromethorphan hydrobromide in

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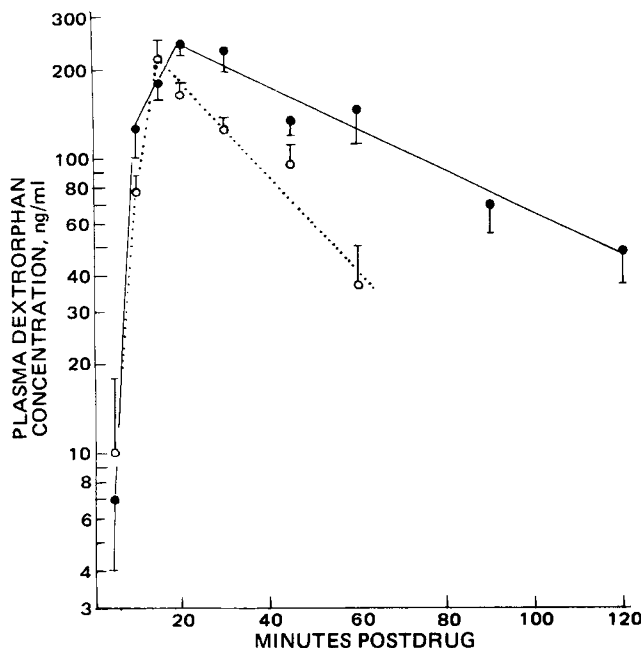

Figure 1—*Concentrations of dextrorphan in the plasma of male (○) and female (●) rats administered dextromethorphan hydrobromide (10 mg/kg po). Each point on the graph represents the mean ± SE of 10 female or four to seven male rats. The numbers of male rats were five at 5, 15, 45, and 60 min; seven at 10 and 20 min; and four at 30 min. The two groups differed significantly at 10 and 20 min (*$p < 0.05$*) and 30 and 60 min (*$p < 0.01$*). The data with female rats were reported by Ramachander* et al. *(15).*

humans. This report describes the differences in the plasma half-life of dextrorphan between male and female rats.

EXPERIMENTAL

Materials—Dextromethorphan hydrobromide[1], dextrorphan[1], spectroquality ethyl acetate[2], and analytical reagent grade *n*-hexane[3] were used.

Treatment of Animals—Groups of Sprague-Dawley rats, females (160–200 g) and males (150–200 g), were maintained on a standard laboratory diet for 1 week prior to use. After fasting for 18 hr, they received by stomach tube an aqueous solution of dextromethorphan hydrobromide, 10 mg/kg.

Blood samples were withdrawn into heparinized tubes by cardiac puncture immediately prior to drug administration and at various intervals thereafter (Fig. 1). Plasma was separated by centrifugation and analyzed for dextrorphan. Plasma obtained from one rat was employed to obtain one experimental dextrorphan concentration. Each reported value was derived from 10 female or four to seven male rats.

Determination of Dextrorphan in Plasma—The previously reported procedure (6) was employed. Plasma (3.0 ml) was adjusted to pH 9.5 by the addition of about 0.6 ml of saturated sodium carbonate solution and extracted with 15 ml of ethyl acetate. The mixture was centrifuged, and 12 ml of the organic layer was shaken with 3.0 ml of 1.0 *N* HCl. The acid layer was separated, and its fluorescence was measured in a fluorescence spectrophotometer[4]. The fluorescence and excitation wavelengths were 310 and 280 nm, respectively, and the slit dials were set to a bandpass of 8 nm for excitation and 5 nm for fluorescence. The dextrorphan concentration in the samples was read from a calibration curve whose validity was determined by including three to four known concentrations of the drug added to rat plasma with each series of unknowns.

Plasma half-lives were estimated by obtaining the slope of the line by the method of least squares, when ln C_p is plotted *versus* t, and dividing the absolute values of the slope into 0.693 (7).

[1] Hoffmann-La Roche, Nutley, N.J.
[2] Matheson, Coleman and Bell, Norwood, Ohio.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.
[4] Perkin-Elmer model MPF-3.

RESULTS AND DISCUSSION

After oral administration of dextromethorphan hydrobromide (10 mg/kg) to male rats, dextrorphan appeared in plasma in 5 min (10 ng/ml), reached peak levels at 15 min (213 ng/ml), and declined thereafter (Fig. 1). The previously reported (6) average level of dextrorphan in the plasma of female rats was 7 ng/ml at 5 min, attained a maximum of 245 ng/ml at 20 min, and fell thereafter. The plasma dextrorphan concentrations in the female rats were significantly higher at 10 and 20 min ($p < 0.05$) and at 30 and 60 min ($p < 0.01$) than in the male rats; the average differences at these sampling times were 50, 81, 103, and 108 ng/ml, respectively. The plasma half-life of dextrorphan was 40 min in the female rats and 18.5 min in the males.

The observed differences in the plasma half-lives of dextrorphan between male and female rats are probably attributable to sex-dependent differences in glucuronidation, but the contribution of other sex-related factors in drug metabolism may be involved. Female rats are known to metabolize xenobiotics more slowly than male rats (8). The ability of hepatic microsomes to form *O*-aminophenol glucuronide *in vitro* was four times greater in male rats than in female rats (9). Progesterone glucuronide formation *in vitro* was faster and occurred in significantly greater quantities in male rats than in females (10).

Substantial sex-dependent differences in the metabolism of drugs also occur in humans. The metabolism of nicotine was reported (11) to differ between male and female smokers. Serum aspirin esterase activity was significantly higher in men than in women (12). The half-life of antipyrine was 30% longer in males than in females (13). In light of such evidence, it appears noteworthy to determine the clinical significance of the observed differences in the plasma half-lives of dextrorphan between male and female rats. At a dose of 2.0 mg/kg, this metabolite was reported (14) to possess antitussive activity comparable to that of dextromethorphan in the dog.

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