in 15-mL fractions 32–55 (**4b**) or 40–57 (**4e**). The 5′-phosphorothioates were both isolated as white freeze-dried solids in 42% yield.

The compounds were further purified by preparative paper chromatography in solvent III[4] and recovered (>95%) after lyophilization as the monoammonium salts.

2-Ethylthioadenosine 5′-phosphorothioate (4f) was prepared from **1f** in a manner similar to the above and was isolated after ion-exchange and paper chromatography in 26% yield.

Synthesis of 5′-*O*-Sulfamoyl Derivatives 5a,b,e. 2′,3′-*O*-Isopropylidene nucleoside (**2a**,**b**[6] or **2e**) was dissolved in 1,2-dimethoxyethane (50–70 mL) and treated with NaH. After 0.5–1 h of stirring, sulfamoyl chloride in 1,2-dimethoxyethane (20 mL) was added dropwise over 20 min and the suspension was stirred for a further 4 h at 25 ° C. The relative quantities of reactants used are given in Table III. Absolute EtOH (10 mL) was added and after stirring for 10 min the mixture was evaporated to dryness. The residue was suspended in water, neutralized (0.1 M HCl), and extracted first with *n*-hexane and then with EtOAc (in the case of the methylthio derivative the residue was extracted with hexane before being dissolved in 50% EtOH and neutralized with HCl; this solution was evaporated to dryness and the dried solid residue was extracted with EtOAc). Solvent was removed from the EtOAc extract in vacuo and the residue was treated with 20% (50% for **5e**) HCOOH for 48 h. Volatiles were removed and the product was crystallized from water.

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Studies on Drug Metabolism by Use of Isotopes. 23.[1] Metabolic Study of 1-Butyryl-4-cinnamylpiperazine in the Rat during Development of Tolerance by Using Two Kinds of Deuterium-Labeled Forms

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Mass fragmentographic determination of the plasma, brain, and liver levels of 1-butyryl-4-cinnamylpiperazine (I) and its metabolites in tolerant and nontolerant rats was carried out by using two kinds of deuterium-labeled compounds. 1-Butyryl-4-[α-d_2]cinnamylpiperazine (I-d_2) was used to develop tolerance and 1-butyryl-4-[arom-d_5]cinnamylpiperazine (I-d_5) to clarify the metabolic fate of I after I-d_2 administration. The present method allowed the clarification of the metabolism of I-d_2 and I-d_5 independently. In tolerant rats, the brain and liver levels were lower for I-d_5 and higher for I-d_5 metabolites than in nontolerant rats. There was no significant difference in the plasma levels of I-d_5 between tolerant and nontolerant rats. Our data seem to suggest that development of tolerance to I results from a more rapid metabolism of the drug due to hepatic enzyme induction.

Several methods have been reported to investigate the effects of repeated administration of a drug on its own metabolism. For example, by spectrophotometry[2,3] the plasma half-lives of drug repeatedly and singly administered to experimental animals have been compared. The plasma, brain, and tissue levels[4-8] and the urinary excretion[9] of drugs and their metabolites in singly and repeatedly administered animals have been determined by a radioactive isotope tracer technique. By these methods, however, simultaneous determination of the metabolic fate of a drug repeatedly administered and that of the same drug finally administered cannot be performed.

The use of stable isotopes together with mass spectrometry has provided a specific and sensitive method for the simultaneous determination of labeled and unlabeled drug in plasma.[10] This method is of great value in the study of steady-state pharmacokinetics of drugs.[11]

In this paper we propose the following experimental method of using a stable isotope tracer technique in which the metabolism of drug repeatedly administered and the metabolism of the same drug finally administered can be clarified simultaneously. The main idea is to use two forms of stable isotope-labeled drug, e.g., drug A-d_2 and drug A-d_5. After repeated administration of drug A-d_2, a single dose of drug A-d_5 is given. Quantitative determination of drug A-d_2 and drug A-d_5, and their respective metabolites, is then performed simultaneously by a gas chromatograph–mass spectrometer–multiple ion detector (GC–MS–MID). As internal standard for the GC–MS–MID measurements, unlabeled compounds of the drug and its

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Table I. Stability of Labeling in 1-Butyryl-4-[α-d_2]cinnamylpiperazine

Sample	Incubation time, h	M − 1⁺/M⁺	M − 2⁺/M⁺
Nontreated		0.154 ± 0.002[a]	0.065 ± 0.002[a]
Dissolved in phosphate buffer (pH 7.4)	24	0.155 ± 0.001[a]	0.066 ± 0.002[a]
	48	0.158 ± 0.000	0.069 ± 0.001
	96	0.157 ± 0.003	0.067 ± 0.002
Dissolved in 1 N HCl	24	0.163 ± 0.002[a]	0.064 ± 0.002[a]
	48	0.160 ± 0.000	0.069 ± 0.003
	96	0.159 ± 0.002	0.071 ± 0.001
Dissolved in 1 N NaOH	24	0.160 ± 0.000[a]	0.082 ± 0.002[a]
	48	0.167 ± 0.009	0.072 ± 0.004
	96	Decomposed[b]	Decomposed[b]
In vivo urine[c]		0.151 ± 0.007[d]	0.072 ± 0.005[d]

[a] Mean ± SD of three measurements. [b] In this condition 1-butyryl-4-cinnamylpiperazine was completely decomposed into 1-cinnamylpiperazine, etc. [c] After administration of I-d_2 to rats, the 24-h urine was collected and analyzed for unchanged I-d_2. [d] Mean ± SD of three experiments.

metabolites are used. In this way it is possible to investigate how the metabolism of the drug was affected by the repeated administration of the same drug.

It has been shown that 1-butyryl-4-cinnamylpiperazine (I)[12] possesses a good analgesic activity.[13] However, repeated administration of a larger dose of I to rats easily developed tolerance to analgesic activity of this compound.[14] Metabolic studies of I in rats and some experimental animal species were also carried out by using GC[15] and radioactive isotope[16] and stable isotope[17,18] tracer techniques. Main metabolites determined by these methods were 1-butyryl-4-(4-hydroxycinnamyl)piperazine (II), 1-cinnamylpiperazine (III), and 1-(4-hydroxycinnamyl)piperazine (IV). It is, therefore, interesting to know how the development of tolerance correlates with the metabolism of I.

In the present study, the effect of repeated administration of I on its own metabolism was demonstrated. Labeled drugs A-d_2 and A-d_5 were 1-butyryl-4-[α-d_2]-cinnamylpiperazine (I-d_2) and 1-butyryl-4-[arom-d_5]-cinnamylpiperazine (I-d_5), respectively. I-d_2 was used to develop tolerance and I-d_5 to clarify the metabolic fate of I after repeated administration of I-d_2. The plasma, liver, and brain levels of I-d_2 and I-d_5 and their respective metabolites in tolerant rats were determined.

Results and Discussion

The present technique was successfully employed for the quantitative determination of I-d_2 and I-d_5 and their respective metabolites separately in the plasma, brain, and liver.

Considerations for GC–MS–MID Analysis. In the preliminary experiments, we carried out fundamental investigations for the practical application of the present technique. Mass spectra of equimolar mixtures of I and I-d_2 and of I and I-d_5 demonstrated that molecular ions at m/e 272:274 (5.7% Σ_{50}) and m/e 272:277 (5.0% Σ_{50}) formed ion clusters of practically equal intensity. This indicates that there was neither isotope effect nor deuterium elimination during the formation of the molecular ion. To measure the relative abundance, therefore, the molecular ions of I, I-d_2, and I-d_5 were selected as the most suitable ions.

As the mass spectra of methyl derivatives of the metabolites of I gave relatively abundant molecular ions (Me-III, 14.4% Σ_{50}; Me_2-IV, 14.5% Σ_{50}; Me-II, 8.8% Σ_{50}), the quantitative determination of the metabolites was performed by measuring these ions in the subsequent experiment.

We found a strict linearity between the peak height ratio and the molar ratio of I and its labeled compounds. This warranted the use of the standard curves for quantitative analysis of I-d_5 and I-d_2 present concomitantly.

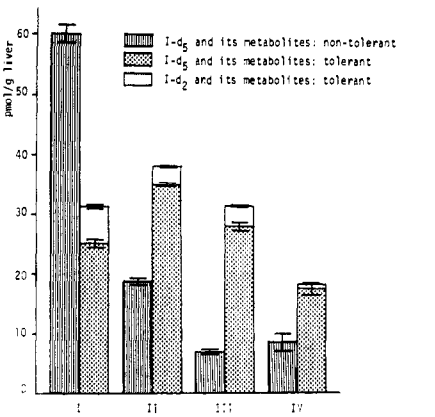

Figure 1. The liver levels of 1-butyryl-4-[α-d_2]cinnamylpiperazine (I-d_2), 1-butyryl-4-[arom-d_5]cinnamylpiperazine (I-d_5), and their metabolites in nontolerant and tolerant rats. I, 1-butyryl-4-cinnamylpiperazine; II, 1-butyryl-4-(4-hydroxycinnamyl)-piperazine; III, 1-cinnamylpiperazine; IV, 1-(4-hydroxycinnamyl)piperazine. Values represent the mean ± SD of $N = 3$. The statistical significance of differences in levels of I-d_5 and its metabolites in the two groups was obtained according to Student's t test ($p < 0.001$ for I–IV).

The quantitative determination of metabolites was carried out on the assumption that the relationship between the peak height ratio and the molar ratio of I and I-d_2 or I-d_5 holds true for the relationship between the peak height ratio and the molar ratio of the metabolites of I and I-d_2 or I-d_5.

There is a possibility that deuterium atoms in the molecule exchange with protium atoms during tracer experiments. However, results shown in Table I indicate that deuterium atoms in I-d_2 were stable in a buffer, strongly basic or acidic solution, and during tracer experiments in vivo. The stability of deuterium atoms of the I-d_5 form had already been established.[19]

Liver, Brain, and Plasma Levels of I-d_5 and I-d_2 Metabolites in Tolerant and Nontolerant Rats. The liver, brain, and plasma levels of I metabolites were determined at a time point of 20 min of peak blood levels. Figure 1 shows that the liver levels of unchanged I-d_5 were much lower in tolerant rats than in nontolerant rats, while the levels of metabolites were higher in tolerant rats than in nontolerant rats. One of the greatest advantages of the present method is that measurements of I-d_2 administered to develop tolerance are performed simultaneously with measurements of I-d_5 administered after the tolerance development. I-d_2 was also found in the liver as in the forms of unchanged and metabolites II, III, and IV.

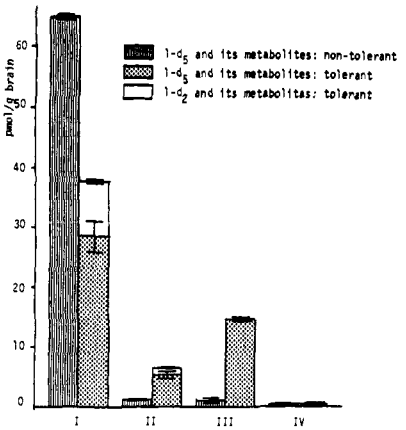

Figure 2. The brain levels of 1-butyryl-4-[α-d_2]cinnamylpiperazine (I-d_2), 1-butyryl-4-[arom-d_5]cinnamylpiperazine (I-d_5), and their metabolites in nontolerant and tolerant rats. I, 1-butyryl-4-cinnamylpiperazine; II, 1-butyryl-4-(4-hydroxycinnamyl)piperazine; III, 1-cinnamylpiperazine; IV, 1-(4-hydroxycinnamyl)piperazine. Values represent the mean ± SD of $N = 3$. The statistical significance of differences in levels of I-d_5 and its metabolites in the two groups was obtained according to Student's t test ($p < 0.001$ for I–III and NS for IV).

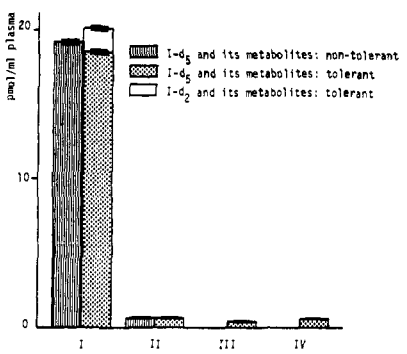

Figure 3. The plasma levels of 1-butyryl-4-[α-d_2]cinnamylpiperazine (I-d_2), 1-butyryl-4-[arom-d_5]cinnamylpiperazine (I-d_5), and their metabolites in nontolerant and tolerant rats. I, 1-butyryl-4-cinnamylpiperazine; II, 1-butyryl-4-(4-hydroxycinnamyl)piperazine; III, 1-cinnamylpiperazine; IV, 1-(4-hydroxycinnamyl)piperazine. Values represent the mean ± SD of $N = 3$. The statistical significance of differences in levels of I-d_5 in the two groups was obtained according to Student's t test (NS, $0.5 < p < 0.4$).

Figure 2 shows that a histogram profile of the brain levels of unchanged I and its metabolites was similar in pattern to that of the liver levels. In the brain, however, I-d_2 was found only in the forms of unchanged I and metabolite II. Furthermore, the amount of metabolite III was much higher in tolerant rats than in nontolerant rats. The results seem to indicate that three metabolites in question do not possess analgesic activity. When one considers the relationship between the tolerance development and the analgesic activity of I, it is interesting to note that the sum of unchanged I-d_5 and I-d_2 levels of tolerant rats was still lower than the I-d_5 level of nontolerant rats.

Figure 3 shows that there was no appreciable difference between tolerant and nontolerant rats in the plasma levels of I-d_5 and its metabolites. In the plasma, the relative levels of metabolites II, III, and IV to unchanged I were very low. There was only a small amount of unchanged I-d_2 and no detectable amount of metabolites of I-d_2. The data shown in Figure 4 (see paragraph at end of paper regarding supplementary material) are the time course of total drug levels obtained from measurements of total

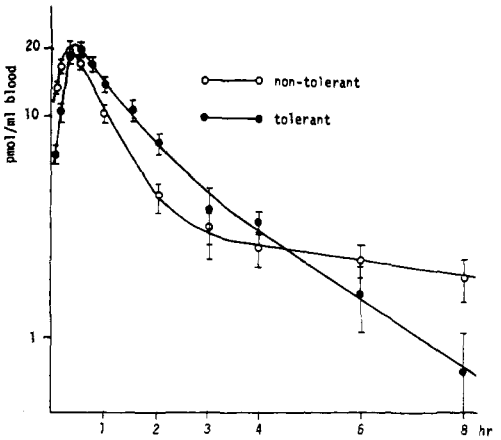

Figure 4. Semilogarithmic plot of ^{14}C activity in the blood of tolerant and nontolerant rats vs. time after subcutaneous administration of 1-butyryl-4-[γ-^{14}C]cinnamylpiperazine. Values represent the mean ± SD of $N = 3$. The statistical significance of differences in levels of ^{14}C activity in the two groups at 1, 2, and 8 h was obtained according to Student's t test ($p < 0.02$ for 1 and 2 h and $p < 0.05$ for 8 h).

radioactivity in the blood. When I-^{14}C was injected subcutaneously after the repeated administration of nonlabeled I for 3 days (tolerant group), the maximum peak level observed in 20 min was the same as in the nontolerant group. From the data in Figure 3 it is not unreasonable to assume that the radioactivity in the blood represents the blood concentration of unchanged I. In tolerant and nontolerant groups the $t_{1/2}$ of the distribution phase was identical ($t_{1/2} = 0.53$ h), whereas the $t_{1/2}$ of the elimination phase was 2.0 h in tolerant rats and 8.9 h in nontolerant rats. The overall elimination curve in tolerant rats is steeper than that in nontolerant rats. One of the reasons for the difference in the $t_{1/2}$ of the elimination phase may be that the rate of metabolism is faster in tolerant rats than in nontolerant rats. It is also possible that faster redistribution of I from the peripheral tissue to the blood in tolerant rats may contribute to the difference in the slope of the elimination phase to some extent (the distribution rate constant for transfer from peripheral to central compartment, k_{21}, is equal to 0.23 in nontolerant rats and 0.69 in tolerant rats).

The fact that I-d_5 was more extensively metabolized by the liver in tolerant rats might be attributable to induction of the drug metabolizing enzymes after repeated administration of I-d_2. It has been shown that aniline hydroxylase activity of the hepatic microsome fraction was induced by repeated administration of I.[14] Since the brain levels of I were lower in tolerant rats than in nontolerant rats, there is a possibility that the development of tolerance may be related to the brain levels of I rather than to a change in the analgesic sensitivity at the cellular level. This view is supported by the finding that concomitant administration of I and ethionine, which is known to inhibit the microsomal enzyme induction, did not develop tolerance.[14] It has also been reported that analgesic activity of I was reduced by pretreatment of phenobarbital.[14] Our data seem to suggest that the development of tolerance to I results from a more rapid metabolism of the drug due to hepatic enzyme induction, although functional tolerance in the central nervous system cannot be entirely excluded.

Experimental Section

Instrumentation. Mass spectra were obtained on a Shimadzu LKB-9000 gas chromatograph–mass spectrometer (GC–MS). Mass fragmentographic measurements were made with GC–MS equipped with a Shimadzu high-speed multiple ion detector–peak

matcher 9060S (GC–MS–MID). The GC conditions were column, 1 m by 3 mm i.d. glass column packed with 1.5% SE-30 on Chromosorb W (60–80 mesh); flash heater, 250 °C; helium flow rate, 20 mL/min. The mass spectrometer conditions were separator, 280 °C; ionization source, 310 °C; ionization energy, 20 eV.

The radioactivity was counted in an Aloka LSC-501 liquid scintillation counter and corrections for quenching were achieved by using external standard. The liquid sample was dissolved in 10 mL of scintillation solvent [dioxane–toluene–methyl cellosolve (75:15:10, v/v) containing 100 g of naphthalene, 4 g of 2,5-diphenyloxazole, and 0.4 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per liter]. The blood and tissue homogenate samples were placed in a small piece of cotton and combusted in an Aloka ASC-112 automatic oxidizer.

Labeled Compounds. 1-Butyryl-4-[γ-^{14}C]cinnamylpiperazine (I-^{14}C) hydrochloride (3.73 μCi/mg) was prepared in this laboratory.[16] 1-Butyryl-4-[arom-d_5]cinnamylpiperazine (I-d_5) hydrochloride was prepared from [arom-d_5]benzaldehyde (99 atom % D) in this laboratory.[17] 1-Butyryl-4-[α-d_2]cinnamylpiperazine (I-d_2) hydrochloride was prepared in a similar manner to the synthesis of I-d_5. Two deuterium atoms were introduced into the α position of cinnamyl alcohol by the reduction of cinnamic acid with $NaBD_4$ (98 atom % D) in D_2O (99.75 atom % D). I-d_2 hydrochloride was recrystallized from acetonitrile to give colorless crystal, mp 203–204.5 °C. Anal. ($C_{17}H_{23}D_2N_2OCl$) C, N; H + D: calcd, 8.11; found, 8.10.

Nonlabeled Compounds. 1-Butyryl-4-(4-hydroxycinnamyl)piperazine (II) hydrochloride,[15] 1-cinnamylpiperazine (III) dihydrochloride,[12] and 1-(4-hydroxycinnamyl)piperazine (IV) dihydrochloride monohydrate[15] were prepared in this laboratory.

Relationship between the Peak Height Ratio and the Molar Ratio of 1-Butyryl-4-cinnamylpiperazine and Its Labeled Compounds. Standard mixtures containing a constant amount (50 μg) of I hydrochloride and variable amounts (0.1–50 μg) of I-d_5 hydrochloride and/or I-d_2 hydrochloride [I-d_5/I, I-d_2/I, and (I-d_5 + I-d_2)/I] were prepared and subjected to GC–MS–MID. The MID was focused at the molecular ions of m/e 272, 274, and 277. Contribution of I, I-d_5, or I-d_2 to the other channels was corrected. The relationships between the corrected peak height ratio (y) and the molar ratio (x) of I and its labeled compounds were $y = 0.995x + 0.003$ for I-d_5/I, $y = 0.940x - 0.006$ for I-d_2/I, $y = 1.000x + 0.001$ for I-d_5/I in (I-d_5 + I-d_2)/I, and $y = 0.960x + 0.011$ for I-d_2/I in (I-d_5 + I-d_2)/I, and the corresponding correlation coefficients were 0.998, 0.998, 1.000, and 0.999, respectively.

Recovery of 1-Butyryl-4-[γ-^{14}C]cinnamylpiperazine and Its Metabolites. A male Wistar albino rat, weighing ca. 150 g, was injected sc with a single dose of 20 mg/kg of I-^{14}C hydrochloride. After 20 min, the rat was sacrificed; the whole liver (ca. 6.5 g) and brain (ca. 1.2 g) were removed and homogenized with 0.1 N HCl (10 mL for the liver and 3 mL for the brain). The homogenate (0.5 mL) was placed in a small piece of cotton, combusted, and subjected to liquid scintillation counting. The remaining homogenate was extracted with Me_2CO–H_2O (3:1, v/v, 25 mL for the liver and 10 mL for the brain). A 0.5-mL aliquot of each extract was counted in 10 mL of scintillation solution. Recovery experiments by this method demonstrated that the extraction of I and its metabolites from tissues was efficient (86.7 ± 2.3% for the liver and 93.0 ± 1.1% for the brain).

Stability of Labeling in 1-Butyryl-4-[α-d_2]cinnamylpiperazine. I-d_2 hydrochloride (1 mg) was dissolved in 10 mL of phosphate buffer (pH 7.4) solution, in 10 mL of 1 N NaOH or 10 mL of 1 N HCl, and the solution was kept at 37 °C in an incubator. The solution (1 mL) was taken in 24, 48, and 96 h and extracted with AcOEt (5 mL) at pH 11. The AcOEt extract was subjected to GC–MS–MID, and the relative abundances of M − 1 (m/e 271) and M − 2 (m/e 270) ions to the molecular ion (m/e 272) were determined.

Three male Wistar albino rats, each weighing ca. 150 g, were injected sc with a single dose of 20 mg/kg of I-d_2 hydrochloride. Urine samples were collected for 24 h. After extraction with AcOEt at pH 11, the AcOEt was evaporated in vacuo and the extract was subjected to TLC (Merck, silica gel 60 F-254). The developing solvent system was benzene–Me_2CO–MeOH–28% NH_4OH (100:24:12:2, v/v). The zone corresponding to I-d_2 was scraped off and eluted with MeOH. The MeOH eluate was then subjected to GC–MS–MID.

Metabolic Fate of 1-Butyryl-4-cinnamylpiperazine in Tolerant and Nontolerant Rats. Six male Wistar albino rats, each weighing ca. 150 g, were divided into tolerant and nontolerant groups. Rats in the tolerant group received a total daily dose of 100 mg/kg of I-d_2 hydrochloride for the first 3 days, i.e., sc injection of 20 mg/kg and ip injection of 80 mg/kg 30 min after the sc injection. This dosage schedule was the same as used to investigate the tolerance to the analgesic activity of I by a "pressure method[20]". Analgesic activity was determined 30 min after the sc injection of 20 mg/kg of I, and then 80 mg/kg of I was injected to develop tolerance. By this dosage schedule, rats were made tolerant completely on the fourth day.[14] Rats in the nontolerant group were under repeated daily treatment with saline alone during this period. On the fourth day, rats in both groups were injected sc with a single dose of 20 mg/kg of I-d_5 hydrochloride. After 20 min, the rats were sacrificed with Et_2O and from each rat 8 mL of a blood sample was collected from the abdominal aorta with a heparinized syringe. The liver and brain were then removed and weighed. The plasma sample was obtained by centrifuging the blood at 3000 rpm for 5 min in a heparinized tube. The whole liver (ca. 6.4 g) and brain (ca. 1.2 g) were homogenized with 0.1 N HCl (10 mL for the liver and 3 mL for the brain) and extracted five times with Me_2CO–H_2O (3:1, v/v, 25 mL for the liver and 10 mL for the brain). As internal standard 5–50 μg of unlabeled I, II, III, and IV was added to one-half aliquot of the liver or brain extract or the plasma. After extraction with AcOEt at pH 11, the AcOEt was evaporated in vacuo and the extract was subjected to TLC twice. The developing solvent systems were benzene–Me_2CO–MeOH–28% NH_4OH (100:24:12:2, v/v) and benzene–Me_2CO–MeOH–28% NH_4OH (20:5:20:2, v/v). UV positive zones corresponding to the metabolites were scraped off and eluted with MeOH. The MeOH eluate was concentrated to about 50 μL under a stream of dry nitrogen. Metabolites II, III, and IV were methylated with diazomethane according to the method of Terayama.[15] The sample (1–2 μL) thus obtained was subjected to GC–MS–MID. The MID was focused at the following molecular ions: m/e 272, 274, and 277 for I; m/e 302, 304, and 306 for Me-II; m/e 216, 218, and 221 for Me-III; and m/e 246, 248, and 250 for Me_2-IV.

Levels of Total Radioactivity in Plasma. Six male Wistar albino rats, each weighing ca. 150 g, were divided into tolerant and nontolerant groups. Rats in both groups received I as described above. Unlabeled I and I-^{14}C were used in place of I-d_2 and I-d_5, respectively. The blood sample (20 μL) was collected from the tail vein, placed in a small piece of cotton, combusted, and subjected to liquid scintillation counting.

Supplementary Material Available: Explanatory material for Figure 4, mass spectra of equimolar mixtures of I and I-d_2 and of I and I-d_5 (Figure 5), mass spectra of methyl derivatives of the metabolites of I (Figure 6), and the relationships between the peak height ratio and the molar ratio of I and its labeled compounds (Figure 7) (3 pages). Ordering information is given on any current masthead page.

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Tricyclic Aryl-Substituted Anticoccidial Azauracils[1]

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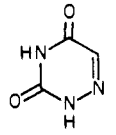
Syntheses of tricyclic aryl-substituted 6-azauracils are described. These compounds showed anticoccidial activity when tested against *Eimeria tenella* and *E. necatrix*. Compound activity was correlated with the chemical shift of the azauracil ring proton. No correlation existed between activity and compound lipophilicity. One of the compounds, 2-(11-oxo-6,11-dihydrodibenzo[*b*,*e*]thiepin-3-yl)-*as*-triazine-3,5(2*H*,4*H*)-dione (**23**), was tested extensively against *E. tenella* and *E. brunetti* both in vivo and in vitro. Compound **23** controlled mortality due to *E. tenella* at 62 ppm, and it afforded protection as measured by weight gain at 31 ppm. Compound **23** afforded little protection against *E. brunetti*. In vitro experiments with **23** showed that it exerted a coccidiostatic effect.

Weak anticoccidial activity (500 ppm) has been reported for 6-azauracil (1).[2,3] Further modification of 6-azauracil resulted in a series of 1-benzyl compounds **2**.[4] The most



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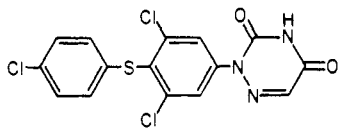

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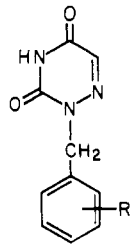

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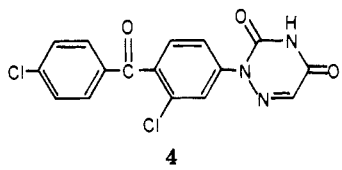

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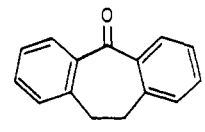
active compounds of this series were substituted with electron-withdrawing substituents in the meta position of the phenyl ring. Azauracils substituted in the 1 position with an aryl substituent were claimed in the patent literature and one of the most active of these compounds was the diphenylthioether-substituted derivative **3**.[5] A detailed report of the anticoccidial activity of **3** against *Eimeria tenella*, *E. necatrix*, and *E. brunetti* has been published.[6] The benzophenone-substituted derivative **4** has been reported effective against major *Eimeria* species at 15 ppm.[7]

The increased activity of **3** and **4** led us to attempt to combine common structural features from these molecules and to incorporate these features into other modified azauracils. A feature common to the aryl moiety of **2–4** is the presence of a meta substituent. Furthermore, there is present in the aryl moiety of **3** and **4** an ortho effect arising from 3,4-disubstitution. This buttressing effect is expected to produce a distortion from planarity of the two aryl rings and, hence, an alteration of the normal electronic contribution of the 4-substituent. Yet another property associated with nonplanarity is the alteration of the normal lipophilicity contributions associated with individual substituents.[8]

By bridging the two phenyl rings found in the azauracil substituent of **3** and **4**, one may produce a variety of tricyclic azauracil substituents which could in theory exhibit all of the substituent properties detailed above. Accordingly, the tricyclic systems **5–8** could serve as interesting substituents for azauracil modification. An important feature in tricyclics **5–8** is that they are nonplanar.



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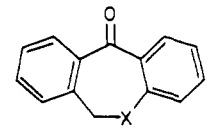
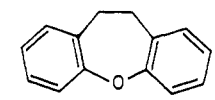


6, X = S
7, X = O



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Chemistry. The general scheme of Slouka[9,10] was modified to prepare 1-substituted 6-azauracils. This method (Scheme I) used the condensation of a diazonium salt with cyanoacetylurethane to give a hydrazone **11**, which was cyclized to a cyano-substituted azauracil **12**. The further transformation of **12** to **14** involved hydrolysis and decarboxylation steps. Since our diazonium salts were quite insoluble in water, an acetic acid–water solvent mixture was used as described in the preparation of **3**.[6] Thioglycolic acid was used to effect the decarboxylation of the azauracil-5-carboxylic acid.[11] In all cases the intermediates **11–13** were used without extensive purifica-

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