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METABOLISM OF THE ANTICONVULSANT 10,11-DIHYDRO-5H-DIBENZO[a,d]CYCLOHEPTENE-5-CARBOXAMIDE—I

METABOLIC FATE OF [^{14}C]CYHEPTAMIDE IN ANIMALS AND MAN*

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Abstract—The metabolic fate of the anticonvulsant cyheptamide (AY-8682) has been investigated in animals and man. In the rat [$^{14}CONH_2$]cyheptamide is rapidly metabolized. Hydroxylation and conjugation are the major modes of metabolism. Cleavage of C-5 from the dibenzo-cycloheptadiene ring does not occur. 10,11-Dihydro-10,5-(epoxymethano)-5H-dibenzo[a,d]cyclohepten-13-one ("lactone") was isolated and fully characterized from acid-hydrolyzed urine samples of rat, rabbit, dog and man. The "lactone" is an artifact that arises from the acid-catalyzed transannular lactone formation between the C-10-hydroxyl and the C-5-carboxamide group of the true metabolite, *syn*-10-hydroxy-cyheptamide. Further oxidation is species dependent and in rat and man occurs at C-5 and in the dog and rabbit at C-11. *Syn*-11-hydroxy-"lactone" and 5-hydroxy-"lactone" have been isolated and fully characterized from acid-hydrolyzed rabbit and human urine respectively. Cyheptamide also undergoes aromatic hydroxylation, and two monophenolic metabolites have been isolated. The exact position of attachment of the phenolic hydroxyl group remains to be established.

IN THE course of an investigation of the synthesis of new psychotropic agents,[1] the compound 10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-carboxamide (AY-8682, cyheptamide) was found to possess a high degree of anticonvulsant activity in laboratory animals.[2] This property was eventually confirmed in clinical studies.[3] In conjunction with these investigations, a study of the metabolic fate of [^{14}C]-labeled cyheptamide was carried out in both laboratory animals and man. Data obtained in these studies are reported herewith.

MATERIALS AND METHODS

[^{14}C]*Cyheptamide*

Cyheptamide labeled with [^{14}C] in the carboxamide group was prepared by condensing the corresponding 5-chloro compound with [^{14}C]-labeled AgCN, followed by hydrolysis to the desired amide as described by Davis *et al.*[1] The material had a specific

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activity of $8{\cdot}25 \times 10^6$ dis./min/mg. On thin-layer chromatography (TLC) with isopropanol–toluene (2:8) and exposure to iodine vapors, it was seen to give only one spot (R_f 0·5) which coincided with the single radioactive peak.

Other compounds

Authentic samples of several potential metabolites and their derivatives were prepared by unequivocal chemical synthesis[4] and were used for the identification of the isolated metabolites. The compounds were the *syn-* and *anti*-10-hydroxy derivatives of cyheptamide; 10,11-dihydro-10,5-(epoxymethano)-5H-dibenzo[a,d]cyclohepten-13-one ("lactone"); the *syn-* and *anti*-11-hydroxy derivatives of the "lactone"; 5-hydroxy-"lactone"; 5H-dibenzo[a,d]cycloheptene-5-carboxylic acid and 5H-dibenzo[a,d]-cycloheptene-5-carboxamide (AY-15,613).

Exploratory metabolic study

An albino male rat, weighing approximately 150 g, was injected intraperitoneally with 5 mg/kg of [^{14}C]cyheptamide and immediately placed in an all-glass metabolism cage. Respiratory carbon dioxide and urine were collected over the subsequent 24-hr period. Total radioactivity in the urine was determined by liquid scintillation counting of 0·2 ml in a dioxane fluor using a Nuclear Chicago model 720 counting system. Ether-extractable radioactivity in the urine was also determined. Respiratory carbon dioxide absorbed in 10% NaOH was isolated after precipitation with barium chloride. The $BaCO_3$ was washed and dried and, after plating on stainless steel planchettes, it was counted in a Nuclear Chicago model C115 gas flow counter.

Distribution studies

Rats. Male albino rats, weighing about 150 g, were given by gavage 10 mg/kg/day of cyheptamide for 3 days. On the fourth day each rat received *per os* 5 mg/kg of [^{14}C]cyheptamide, and groups of six animals were killed 30 and 120 min later.* Samples of serum, brain, liver and gastrointestinal (GI) tract (stomach, small and large intestine and their contents) were taken and, after the addition of 100 mg cyheptamide used as carrier, each tissue was homogenized with alcohol–ether (3:1). The radioactivity content, i.e. cyheptamide plus metabolites, was determined in an aliquot of each tissue extract. The remainder was evaporated to dryness and any lipid material was removed by thoroughly triturating the crystalline residue with hexane. The contribution of unchanged cyheptamide was determined from the specific activity of the purified carrier cyheptamide subsequently isolated. The residue was repeatedly crystallized from hot 50% ethanol until the content of radioactivity was constant (see Table 1). Because of the large amounts of lipid impurities, the residue from the GI tract could not be successfully triturated with hexane. It was therefore chromatographed on silicic acid (2 × 40 cm) with chloroform as eluant. Fractions of 100 ml were collected and cyheptamide was eluted in the fifth or sixth fraction. The solvent was removed under reduced pressure and the residue was recrystallized as mentioned above.

Man. [^{14}C]cyheptamide (900 mg; specific activity, $6{\cdot}9 \times 10^3$ dis./min/mg) was

* Experiments based on the quantitative colorimetric determination of 10-hydroxylated metabolites of cyheptamide have indicated that its subchronic administration to rats does not result in stimulation of its own metabolism.

administered orally as 3 dry powder capsules, to three healthy male volunteers. Blood and urine samples were taken at various times for periods up to 72 hr after the dose. The sera were analyzed for cyheptamide and its metabolites by the procedures used in the rat studies.

Urinary excretion. In a separate study, 10 male rats, each weighing approximately 150 g and housed individually in stainless steel metabolism cages, were given 100 mg/kg of [^{14}C]cyheptamide orally. Urine samples were collected over two successive 24-hr intervals and the total radioactivity content was determined. No attempt was made to measure free cyheptamide by the carrier technique, as preliminary experiments had shown that only traces were present.

Urinary metabolites

Urine samples from rats, dogs, rabbits and men were prepared in the same manner. Urine was diluted with distilled water and HCl was added to make a final concentration of 1·0 N. The conjugates were hydrolyzed by refluxing for 1 hr and, after cooling, the neutral and acidic materials were extracted three times with equal volumes of methylene chloride. The combined methylene chloride extracts were dried over anhydrous Na_2SO_4, reduced to a small volume under vacuum and chromatographed on silica gel (Grace, grade 923). Usually about 2·0 g of extract was chromatographed on a 300 × 20 mm column. Fractions (25 ml) were obtained by successive elution with methylene chloride, chloroform and chloroform–methanol mixtures. Fractions containing significant amounts of a metabolite were combined, dried and the metabolite was purified by repeated crystallization from suitable solvents. The metabolites were characterized by thin-layer chromatography, ultraviolet (u.v.), infrared (i.r.) and nuclear magnetic resonance (n.m.r.) spectroscopy, elemental analysis, and, when possible, by direct comparison with an authentic sample.

In some experiments the conjugates were hydrolyzed with glucuronidase instead of aqueous HCl. Incubations for periods up to 2 hr were carried out at 37° and at pH 4·8 using Glusulase* (Endo Laboratories, 6000 units/ml of urine). The "free" metabolites were extracted with methylene chloride and examined by thin-layer chromatography for the presence of 10-hydroxy-cyheptamide and the "lactone".

RESULTS

Exploratory study

After intraperitoneal injection of [^{14}C]cyheptamide, less than 0·5 per cent of the administered radioactivity was detected in the expired carbon dioxide collected over a 24-hr period. The finding indicates that scission of the bond between ring carbon 5 and the carboxamide carbon does not occur and thus all metabolites of cyheptamide will contain radiocarbon. The urine collected over the same 24-hr period contained approximately 50 per cent of the administered radioactivity. The fact that only traces of radioactivity were extracted with ether indicated that virtually all of the radioactivity excreted in the urine was associated with conjugated metabolites of cyheptamide. In the rat cyheptamide is extensively catabolized.

* Glusulase is an enzyme preparation obtained from snails that is rich in both glucuronidase and sulfatase enzymes.

TABLE 1. TISSUE DISTRIBUTION OF CYHEPTAMIDE AND ITS METABOLITES IN THE RAT*

	Level (μg/g) (μg/ml)							
	Liver		Serum		Brain		GI tract	
	30 min	120 min	30 min	120 min	30 min	120 min	30 min	120 min
Cyheptamide	0·42 (30)†	0·11 (10)	0·10 (24)	0·03 (8)	0·09 (53)	0·03 (18)	10·8 (90)	6·0 (70)
Metabolites	0·99	1·03	0·31	0·33	0·08	0·14	1·2	2·6

* Male albino rats received a daily oral dose of 10 mg/kg of cyheptamide followed by 5 mg/kg of [^{14}C]cyheptamide on day 4; groups of six rats were killed 30 and 120 min after the last dose.

† Values in parentheses represent levels of cyheptamide expressed as per cent of total radioactivity detected in a tissue.

Serum and tissue levels

The results presented in Table 1 indicate that cyheptamide undergoes rapid catabolism in the rat. At 30 min after oral administration of 5 mg/kg of [^{14}C]cyheptamide, only 25 per cent of the radioactivity present in the serum and liver was due to unchanged cyheptamide and this was further reduced to less than 10 per cent at 120 min. These limited data do not permit the estimation of a half-life, but do indicate that it is very short.

The close similarity of the levels of unchanged cyheptamide in serum and brain, both at 30 and 120 min after the dose, suggests the capacity of cyheptamide to readily pass the blood–brain barrier. Most of the radioactivity in the gastrointestinal tract was associated with unchanged cyheptamide, hence representing unabsorbed material. At 30 min this amounted to one-third of the administered dose.

Serum levels of cyheptamide and its metabolites in men are presented in Fig. 1. Peak levels of cyheptamide approaching 10 μg/ml were seen at approximately 2–4 hr after oral administration. In contrast to the results in rats, unchanged cyheptamide

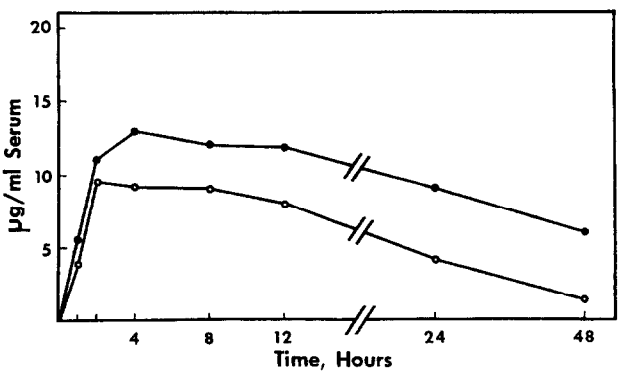

FIG. 1. Serum levels of cyheptamide (○——○) and cyheptamide + metabolites (●——●) in man. Average values for 3 volunteers after a single oral dose of 900 mg.

TABLE 2. COMPARISON OF URINARY EXCRETION PATTERN OF CYHEPTAMIDE AND ITS METABOLITES AFTER ORAL ADMINISTRATION OF [^{14}C]CYHEPTAMIDE IN RAT AND MAN*

Time (hr)	Per cent of dose excreted in urine	
	Rat	Human
0–24	7	29
24–48	0	29
48–72		17
Cumulative	7	75

* The rats received a dose of 100 mg/kg and the humans 900 mg *per os*.

accounted for about two-thirds of the total radioactivity in the serum at this time and, even at 24 hr, it still accounted for 50 per cent of the total. The half-life of cyheptamide in man is thus approximately 18 hr.

Urinary excretion

In rats, after oral administration, urinary excretion of cyheptamide metabolites (Table 2) is essentially complete in 24 hr and accounted for only 7 per cent of the administered dose. In man, the excretion of cyheptamide metabolites in urine was fairly constant over the 3-day period studied and accounted for approximately 75 per cent of the administered dose. The results indicate that cyheptamide is much better absorbed in man than in the rat, although the apparently poor absorption of cyheptamide in the rat may actually be a consequence of a very active biliary excretion.

"*Lactone*"

When a methylene chloride extract of a hydrolyzed rabbit's urine was chromatographed on silica gel, the first material to be eluted with methylene chloride was the "lactone". When chromatographed on TLC plates (silica gel G, $CHCl_3$), the "lactone" gave, after spraying with 30 N H_2SO_4 and heating at 110°, a characteristic pink spot (R_f 0·7). Fractions with the same behavior on TLC were combined, evaporated to dryness and the residue was repeatedly crystallized from ethanol. The "lactone" had the following characteristics: m.p. 170–172°; color reaction (30 N H_2SO_4), λ_{max} 376, 392, 500, 528 mμ; i.r., ν_{max} ($CHCl_3$) 1740 cm^{-1} (C=O); n.m.r., δ ($CDCl_3$) 7·0–7·5 (m, $H_{arom.}$), 5·64 (q, 1H, C-10, $J_{AB} = 2$ Hz, $J_{AX} = 4$ Hz), 3·21 (q, 1H, C-11, *anti*, $J_{AB} = 2$ Hz, $J_{BX} = 18$ Hz), 3·75 (q, 1H, C-11, *syn*, $J_{AX} = 4$ Hz, $J_{BX} = 18$ Hz), 4·69 (s, 1H, C-5). *Anal.* Calcd. for $C_{16}H_{12}O_2$: C, 81·34; H, 5·12. Found: C, 81·19; H, 5·03. The "lactone" was in all respects identical with an authentic sample.[4] Identical material was also isolated from rat, dog and human urine.

Syn-11-*hydroxy*-"*lactone*"

The compound was eluted from a silica gel column with chloroform or chloroform–methanol (90:10). On TLC (silica gel G, $CHCl_3$), when sprayed with 30 N H_2SO_4, it produced a pink spot (R_f 0·2) that turned green on heating. The *syn*-11-hydroxy-"lactone", after repeated crystallization from carbon tetrachloride, had the following

characteristics: m.p. 120–122°; color reaction (30 N H_2SO_4), λ_{max} 625 mμ; i.r., ν_{max} ($CHCl_3$) 1755 cm^{-1} (C=O), 3570 cm^{-1} (OH); n.m.r., δ (DMSO) 7·0–7·5 (m, $H_{arom.}$), 5·68 (d, 1H, C-10, J = 3 Hz), 4·79 (s, 1H, C-5), 4·75 (d, 1H, C-11, J = 3 Hz), 3·40 (s, 1H, C-11, OH; D_2O exchangeable). *Anal.* Calcd. for $C_{16}H_{12}O_3$: C, 76·18; H, 4·80. Found: C, 75·32; H, 5·25.

The compound was identical in all respects with an authentic sample of *syn*-11-hydroxy-"lactone".[4] In no instances did the amount of this substance in the urine of a cyheptamide-treated animal ever exceed 10 per cent of the amount of "lactone" therein. The identical material was also isolated from dog's urine.

When the crude extracts were examined by TLC, a second green spot (initially pink) moving slightly faster than the *syn*-isomer was detected. Since the synthetic *anti*-compound[4] migrates faster than its isomer, it is likely that the second spot corresponds to the *anti*-isomer. Presence of only trace amounts precluded its definitive characterization.

5-hydroxy-"lactone"

During the development of a colorimetric procedure for the determination of levels of "lactone",[5] it was noticed that, on treatment with 30 N H_2SO_4, extracts from human urine gave a cherry-pink color rather than the orange-pink color typical of the "lactone". In addition to the peaks typical for the "lactone", the spectrum contained new peaks at 412 and 564 mμ (Fig. 2). Further, TLC of the "lactonic" fraction isolated from acid-hydrolyzed children's urine on treatment with 30 N H_2SO_4 revealed an elongated spot with the usual orange-pink on the top and the cherry-pink at its base. The mixture was separated by column chromatography on alumina (Woelm, Neutral, activity grade 1). After the "lactone" was eluted with chloroform, the column was washed with chloroform–methanol (90:10). These eluates were combined and evaporated to dryness under reduced pressure.

The residue was boiled with hexane and the supernatant decanted. After recrystallization from carbon tetrachloride–hexane, the material giving an intense cherry red color in 30 N H_2SO_4 had the following characteristics: m.p. 122–124°; color reaction (30 N H_2SO_4), λ_{max} 392, 412, 528, 564 mμ; i.r., ν_{max} ($CHCl_3$) 1748 cm^{-1} (C=O), 3500 cm^{-1} (OH); n.m.r., δ ($CDCl_3$) 6·8–8·0 (m, $H_{arom.}$), 5·71 (q, 1H, C-10, J_{AX} = 2 Hz, J_{BX} = 4 Hz), 3·77 (q, 1H, C-11, *syn*, J_{BX} = 4 Hz, J_{AB} = 18 Hz), 3·20 (q, 1H, C-11, *anti*, J_{AX} = 2 Hz, J_{AB} = 18 Hz), 4·25 (s, 1H, C-5, OH; D_2O exchangeable). *Anal.* Calcd. for $C_{16}H_{12}O_3$: C, 76·18; H, 4·80. Found: C, 76·54, H, 4·91.

The compound was identical with an authentic sample of 5-hydroxy-"lactone".[4] The same material was also isolated from rat urine.

Phenolic metabolites

By stripping the silica gel columns with methanol, some very polar material was eluted. On evaporation, the material crystallized and was purified by recrystallization from methanol. The material isolated from hydrolyzed rabbit urine had the following characteristics: m.p. 213–215°; u.v., λ_{max} (pH 1) 276 mμ (ε = 1610), λ_{max} (0·1 N NaOH) 248 mμ (ε = 8980), 295 mμ (ε = 2374); i.r., ν_{max} (KBr) 1656 cm^{-1} (C=O). *Anal.* Calcd. for $C_{16}H_{15}O_2$: C, 75·87; H; 5·97; N, 5·53. Found: C, 75·15; H, 5·70; N, 5·31.

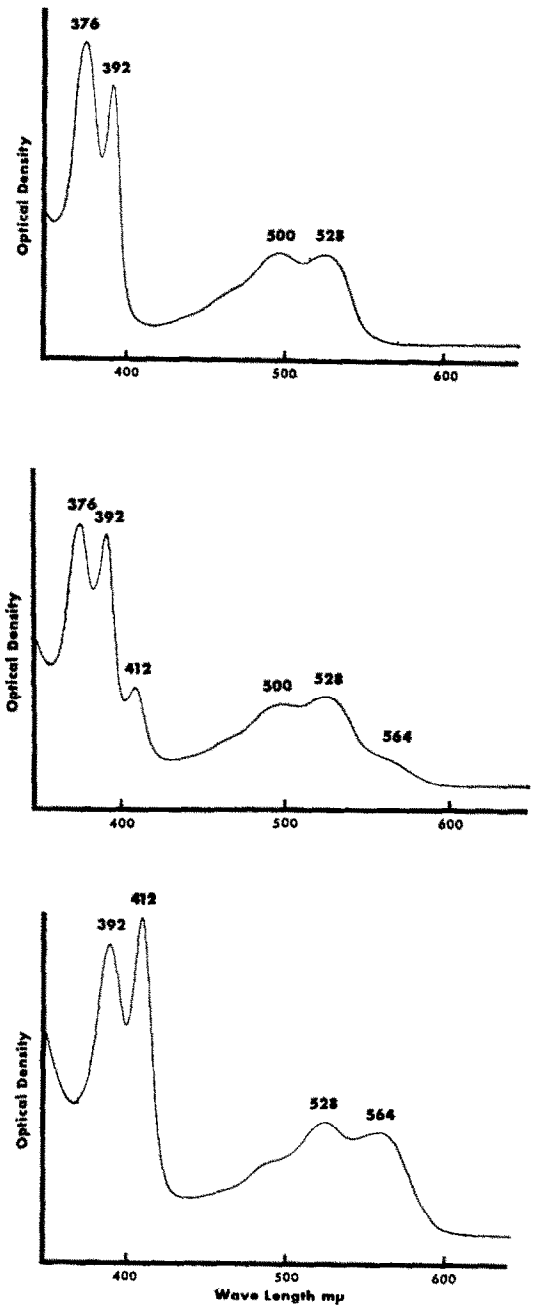

FIG. 2. Visible spectra of "lactone" (top), crude "lactone" fraction from children's urine (middle) and purified 5-hydroxy-"lactone" (bottom) in 30 N H_2SO_4.

The substance is a monophenolic metabolite of cyheptamide. The position of the phenolic hydroxyl remains uncertain, as no authentic samples were available for comparison. When the same procedure was applied to acid-hydrolyzed dog's urine, a different phenol was isolated. After three recrystallizations, the phenol from dog's urine had the following characteristics: m.p. 215–217°; u.v., λ_{max} (pH 1) 282 mμ ($\epsilon = 2142$), λ_{max} (0·1 N NaOH) 243 mμ ($\epsilon = 7071$), 301 mμ ($\epsilon = 3274$); i.r., ν_{max} (KBr) 1663 cm^{-1} (C=O). The material was not suitable for microanalysis and was converted to the acetate by the action of acetic anhydride–pyridine. On recrystallization from chloroform–ether, a monoacetate, m.p. 190–193°, was obtained. *Anal.* Calcd. for $C_{18}H_{17}NO_3$: C, 73·20; H, 5·80. Found: C, 72·97; H, 5·99.

Thus the phenol isolated from acid-hydrolyzed dog's urine is also a monophenolic derivative of cyheptamide, being probably a positional isomer of the phenol from rabbit urine. When human urine was similarly prepared, material identical in all respects to the phenol from rabbit urine was isolated.

Origin of the "lactones"

The 5-carboxamide group of cyheptamide (and of its phenolic metabolites) is very resistant to acid hydrolysis. In marked contrast, on acid hydrolysis of the 10-hydroxylated metabolites of cyheptamide, only transannular lactones were isolated. It is not likely that the 5-carboxamide group undergoes enzymatic deamidation. McMahon and Sullivan[6] have reported that the structurally similar diphenylacetamide is metabolically stable. The 10-hydroxylated metabolites of cyheptamide are probably conjugated with glucuronic acid and excreted with the carboxamide group intact. On acid hydrolysis, the hydroxyl group at position 10 is freed and it promotes by anchimeric assistance (the geometry of a *syn*-10-hydroxy is ideal, cf. Fig. 3) the acid-catalyzed hydrolysis of the previously very stable carboxamide group with resultant lactone

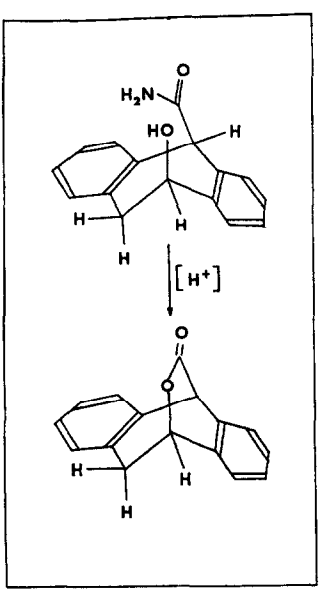

FIG. 3. Acid catalyzed transannular lactonization of *syn*-10-hydroxy-cyheptamide.

formation. The hydroxyl-assisted hydrolysis of amides is well known.[7–9] When authentic *syn*-10-hydroxy-cyheptamide became available by chemical synthesis,[4] its rapid and near quantitative conversion to the "lactone" under mild acid conditions was readily demonstrated. It is during this acid-catalyzed rearrangement that traces of 5H-dibenzo[a,d]cycloheptene-5-carboxylic acid are formed. The traces found in acid-hydrolyzed urines are thus probably artifacts and not of metabolic origin.

Stereochemistry of C-10 *hydroxylation*

The 10-hydroxylated metabolites of cyheptamide are unstable in the presence of acid. Attempts to isolate them from enzymatically hydrolyzed urine were unsuccessful. Glusulase was employed and hydrolysis was carried out at pH 4·8 at 37° for 2 hr. The hydrolysate was examined by TLC in chloroform–methanol (98:2), followed by spraying and heating with 30 N H_2SO_4; only the "lactone" (pink spot, R_f 0·9), but no 10-hydroxy-cyheptamide (pink spot, R_f 0·1), was detected. The fact that synthetic *anti*-10-hydroxy-cyheptamide was recovered unaltered from a simulated hydrolysis medium suggests that the metabolite is the much less stable *syn*-isomer. Synthetic *syn*-10-hydroxy-cyheptamide has been prepared in our laboratories,[4] and was shown to be extremely sensitive to even the slightest traces of acid. Simple recrystallization from hot ethanol resulted in virtually quantitative conversion to the "lactone". Unequivocal proof that enzymatic hydroxylation of cyheptamide at carbon-10 produces the *syn*-isomer awaits the isolation of this metabolite in its conjugated form.

Species differences in cyheptamide metabolism

The excretion pattern for metabolites of cyheptamide in the urine of various species is given in Table 3. In all the examined species, cyheptamide was preferentially hydroxylated at C-10. Additional hydroxylation, however, is species dependent and two distinct patterns appear to exist: in the rat and man the second hydroxyl is introduced at C-5, whereas in the rabbit and dog the second hydroxyl appears on C-11. Differences in phenolic metabolites between species were also noted, but the phenols were not fully characterized.

Anticonvulsant activity of cyheptamide metabolites

Data on the biological activity of the metabolites of cyheptamide were made available through the kind co-operation of Dr. F. Herr of our Department of Pharmacology.

TABLE 3. SPECIES AND SITE OF HYDROXYLATION OF CYHEPTAMIDE

	Hydroxylation site			
Species	C-10	C-10, 11	C-5, 10	C-aromatic
Rat	+		+	*
Rabbit	+	+		+
Dog	+	+		+†
Man	+		+	+

* No attempt was made to isolate phenolic metabolites.
† The phenolic metabolite differs from that isolated from rabbit or human urine and is probably a positional isomer.

All materials were injected in the form of suspensions which were made up with four to five drops of Tween 80 in 10 ml water and administered intraperitoneally. In most cases, the approximate LD_{50} values were determined using 20 mice per compound. For cyheptamide, the LD_{50} was calculated by the method of Litchfield and Wilcoxon,[10] injecting four to five doses into groups of 10 animals each. The protective effect of the compounds against the tonic phase of maximal electroshock seizure (MES) was tested in a way similar to that described by Swinyard *et al.*[11] Groups of 10 animals were pretreated with increasing doses of the compounds and 60 min later an electrical shock (30 mA; 0·2 sec) was applied through corneal electrodes. The ED_{50} values were calculated from the number of protected animals.[10]

The data (Table 4) reveal that all the metabolites or their acid-catalyzed rearrangement products are less active than cyheptamide in their protection of mice against MES seizures.

TABLE 4. BIOLOGICAL ACTIVITY OF CYHEPTAMIDE AND ITS METABOLITES

Compound	Acute toxicity (LD_{50})*	Anticonvulsant activity (MES ED_{50})†
Cyheptamide	630 ± 31	25 ± 1
"Lactone"	>1200	280 ± 30
syn-11-OH-"lactone"	225	46 ± 3
anti-11-OH-"lactone"	1100	72 ± 6
anti-10-OH-cyheptamide	>200	100 ± 6
5-OH-"lactone"	375	41 ± 5

* Usually 20 animals were used per test except in the case of cyheptamide where 50 animals were used[10] to obtain statistical data.

† The mice, in groups of 10, were given graded doses of the compounds and tested for electroshock seizure 60 min later. MES = maximal electroshock seizure.

DISCUSSION

In all species studied, cyheptamide is rapidly metabolized by hydroxylation and conjugation to give water-soluble metabolites which are excreted in the urine. The rate of absorption, the rate of metabolism, and the nature of the metabolites vary from species to species. The metabolic transformations of cyheptamide are summarized in Fig. 4. While additional sites of hydroxylation vary from species to species, in all the species examined, the preferred and major site of hydroxylation was the benzylic carbon-10 of the cycloheptadiene nucleus. Hydroxylation at this site has also been reported for several drugs structurally similar to cyheptamide, e.g. imipramine,[12] amitriptyline[13] and nortriptyline.[14] C-10-hydroxylated metabolites of imipramine are unstable and their acridine-like acid rearrangement products have not been fully characterized. Metabolites of amitriptyline and nortriptyline hydroxylated on C-10 are also prone to acid-catalyzed rearrangements to give ring-contracted, anthracene-like products. The 10-hydroxylated metabolites of cyheptamide differ inasmuch as they do not undergo acid-catalyzed ring contraction. Instead, in the presence of acid, the unique steric relationship between the 10-hydroxyl and the 5-carboxamide is resolved in the formation of a stable transannular lactone. The 5,10- and 10,11-bis-hydroxylated

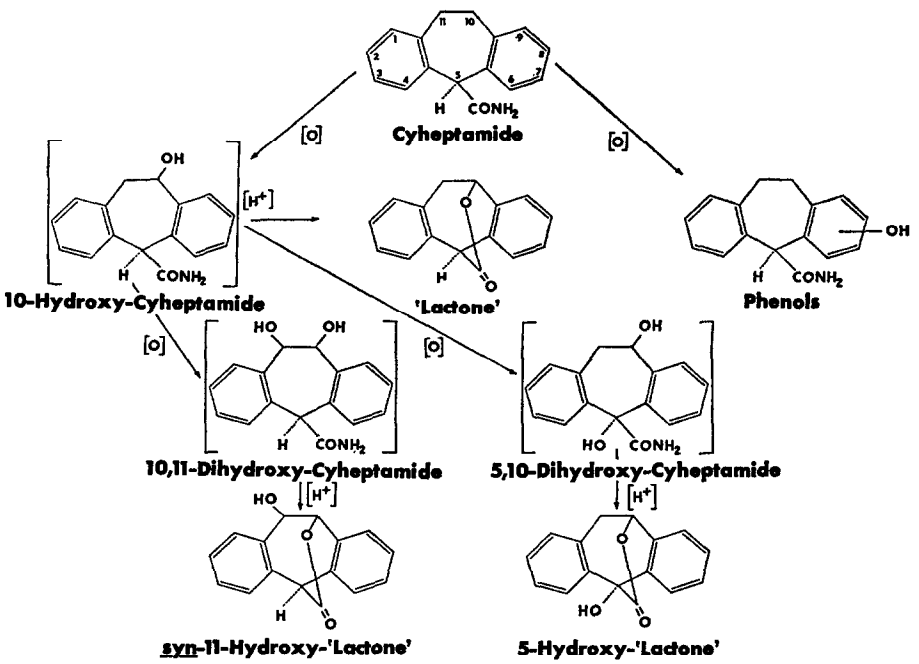

FIG. 4. Metabolic transformations of cyheptamide. Metabolites in parentheses exist only as conjugates and have not been isolated as such.

metabolites behave in the same way. All "lactones" were readily synthesized, thus enabling definite characterization of all 10-hydroxylated metabolites of cyheptamide.

The metabolites of cyheptamide were tested for anticonvulsant activity in mice. All the "lactones" were relatively inactive. This is not surprising since they are deamidated products of 10-hydroxylated metabolites of cyheptamide. *Syn*-10-hydroxy-cyheptamide believed to be the metabolite formed enzymatically, *in vivo*, though prepared by chemical synthesis, proved to be too unstable for pharmacological testing. Its isomer, the *anti*-10-hydroxy derivative, was tested but showed only weak anticonvulsant effect in mice.

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