contamination, it is obvious that other cross contaminations can be detected more easily and to lower levels.

High resolution mass spectra of 1-pentyl and 3-methyl-1-butyl acetates have been reported by Beynon (*1*). The specific rearrangements shown in mass spectral analyses of 2,2-dimethyl-1-propyl esters have been reported in detail (*4*).

Table II summarizes the data on threshold values. While definite differences in threshold values or odor pervasiveness were found among some of the acetates, the total range of values is not particularly large. This is especially apparent in comparing these differences with those obtained with different classes of compounds, which can differ by several orders of magnitude. The general similarity of these values for the acetates is not too surprising, considering the fact that all these compounds have the same number of carbon atoms and the same functional group. There does not appear to be any systematic relation between structural differences and threshold values. The differences in odor pervasiveness reported here could be due to slight traces of impurities undetectable by instrumental means but registered by the olfactory system.

Odor discrimination among the purified compounds listed in Table I was determined by the triangle test ($n = 20$) for 10 of the 36 possible pairs. In all 10 comparisons, odor discrimination between samples was always highly significant ($P < 0.001$). Even though 70% or more of the panel described the characteristic odor of both 1-pentyl and cyclopentyl acetates as fruity-floral, they were able to discriminate between the two compounds at a highly significant level. While most of the compounds were given the general qualitative odor description of fruity-floral, some had other definite odor characteristics. Details of the descriptive odor analyses of these as well as other compounds are being studied and will be reported later.

Table II. Odor Thresholds of Pentyl Acetates

Acetates	Threshold and Standard Deviations, Parts per Billion Parts Water
1-Pentyl	5 ± 0.5
2-Pentyl	2 ± 0.2
3-Pentyl	9 ± 1.0
2-Methyl-1-butyl	5 ± 0.5
3-Methyl-1-butyl	2 ± 0.3
3-Methyl-2-butyl	6 ± 0.3
1,1-Dimethyl-1-propyl	30 ± 5.0
2,2-Dimethyl-1-propyl	4 ± 1.0
Cyclopentyl	21 ± 2.0

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ANIMAL METABOLISM

Further Study on the Metabolism of Labeled 3-Amino-1,2,4-triazole (ATA) and Its Plant Metabolites in Rats

S. C. FANG, SUCHITRA KHANNA, and A. V. RAO

Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore.

3-Amino-1,2,4-triazole-5-C^{14} (ATA) was fed to adult rats at a dosage varying from 1 to 200 mg. per rat, and the excretory pattern, tissue accumulation of C^{14}, and metabolite formation in the urine were determined. There was no significant difference in the per cent recovery of radioactivity in urine and feces in relation to the dosage fed. The formation of ATA-metabolite by rats as per cent of dose decreased as the ATA dosage increased. The rate of elimination of ATA from all tissues was slightly slower with rats fed a 200-mg. dosage as compared with those fed a 1-mg. dosage. Using a mixture of ATA-H^3 and ATA-5-C^{14} and studying the change of H^3/C^{14} ratio in rat-ATA-metabolite indicated that 5-hydrogen atoms in the ring of ATA has been substituted. One of the amino hydrogens in the ATA-metabolite from male rats may be substituted also. Similar studies were carried out in rats using C^{14}-labeled or H^3 and C^{14}-labeled ATA-metabolites isolated from bean plants. The pattern of elimination for metabolite-1 and metabolite-3 differed greatly in adult rats.

Very little study has been done on the metabolism by mammals of 3-amino-1,2,4-triazole (ATA) and its transformation products from plants. In the previous paper (*2*), a study on the excretory pattern, metabolic fate, and tissue residues of ATA-5-C^{14} in rats was reported. This communication reports a further observation on the rate of ATA elimination and metabolite formation in rats on the basis of varying dosages, the nature of ATA metabolism in rats using a mixture of ATA-H^3 and ATA-5-C^{14} as tracer, and the rates of elimination of two ATA transformation products isolated from bean plants.

Materials and Methods

Rate of Excretion and Metabolite Formation in Rat Urine as a Function of Dose. This experiment was designed

to determine whether the excretion rate of ATA through urine and feces, and metabolite formation are a function of oral dose. The ATA-5-C^{14} solution was prepared in the manner described previously (2). With higher dosages, the ATA-5-C^{14} used was the same in each level and made up with the balanced amount of nonlabeled ATA. A number of adult rats of the Wistar strain (4 to 6 months of age) were fed orally. The feeding of the rats, the collection of the urine and feces samples, and the determination of radioactivity and metabolite formation in them were conducted in the same manner (2).

Metabolism of ATA-H^3 and ATA-5-C^{14}. To study the nature of ATA metabolism in rats involving hydrogen atoms of the ATA molecule, a sample of tritium-labeled ATA was prepared by a catalytic exchange procedure as follows. Three hundred milligrams of ATA were dissolved in 0.5 ml. of tritiated water (50 mc.) in a test tube. Thirty milligrams of platinum oxide catalyst were added to the solution, and the tube was sealed. The mixture was heated in an autoclave at 15 pound's pressure for 17 hours. After the tube cooled, the seal was broken and the excess tritiated water was removed under reduced pressure. The ATA-H^3 was dissolved in a small volume of alcohol, filtered, and recrystallized from alcohol. The final product had a specific activity of 1.5 million counts per minute per mg. with the authors' counting system. Further recrystallization of ATA-H^3 in alcohol or in water did not result in a loss of tritium activity. A small aliquot of ATA-H^3 was mixed with a known amount of nonlabeled ATA, and the mixture was dissolved in dry tetrahydrofuran and was acetylated with acetyl chloride (6). The 1-acetyl-ATA-H^3 isolated from this reaction revealed no loss of tritium activity from the original ATA sample. Conversion of 1-acetyl-ATA-H^3 to 3-acetamido-ATA-H^3 showed a loss of from 3 to 5% of tritium activity. Formation of diacetyl derivative of ATA also showed a loss of 5% tritium activity from the original sample. Conversion of diacetyl ATA-H^3 to 3-acetamido-ATA-H^3 resulted in no loss of tritium activity. This observation suggested that in the author's ATA-H^3 sample, more than 90% of the activity was at 5-hydrogen of the ring with the balance in the amino group. The 1-hydrogen atom of the ring was not labeled. The tritium label at 5-hydrogen position will be lost when ATA was refluxed with acetic anhydride for a prolonged period according to the method of Fredrick and Gentile (3).

One hundred milligrams of ATA-H^3 and 0.37 mg. of ATA-5-C^{14} were dissolved in 10 ml. of water, and the ratio of H^3 to C^{14} activity in this mixture was measured according to the method of Prockop and Ebert (5) with a Packard Tricarb Model 314E liquid scintillation spectrometer. The ratio was 11 to 1. A known quantity of this mixture was fed orally to the rats either by means of a stomach tube or by mixing it in a 10% sucrose solution and allowing the rat to drink it from an eye dropper when the feeding was done by a single worker. Both male and female adult rats were used in this study. Some of these rats were dosed as many as five times at two-day intervals between feedings. In each case, the urine sample was collected 24 hours after the administration of ATA and was analyzed for ATA and its metabolite by paper chromatography using 2-propanol·NH_4OH·H_2O (8:1:1) as a developing solvent. ATA and ATA-metabolite spots were eluted separately with water and the H^3:C^{14} ratio of these eluates was determined in the same manner.

Metabolism of ATA-Metabolites Isolated from Bean Plants in the Rats. There is nothing known about the manner in which ATA plant metabolites are metabolized and eliminated by mammals. For this reason, samples of ATA metabolites from bean plants were prepared either using ATA-5-C^{14} or a mixture of ATA-H^3 and ATA-5-C^{14} as starting materials in the following manner. Aqueous solution (0.1 ml.) containing 50 to 500 μg. of labeled ATA was pipetted into small test tubes. Two week old bean plants were cut about 2 inches below the primary leaves, and the cut ends were immediately inserted in the test tubes to take up the ATA solution. After complete absorption of ATA solution, each tube was rinsed with a few drops of water which were also allowed to be taken up completely by bean plants. Finally, all test tubes were filled with water, and the bean plants were maintained under this condition in the greenhouse for 5 days. The leaves were removed and ground in a Waring Blendor with sufficient volume of 50% ethanol. The homogenate was filtered, and the residue was washed several times with a small volume of 50% ethanol. The filtrate and washings were combined and extracted several times with benzene to remove the coloring matters. No radioactivity was found in the benzene extracts. The clear yellowish alcoholic solution was passed directly through a Dowex-50 column in H^+ form according to the method of Carter and Naylor (1). All radioactivity was retained in the column. After the column was thoroughly rinsed with water, the column was eluted with approximately 1500 ml. 0.1N HCl, followed with 500 ml. of 0.5N HCl, and then 1500 ml. 1N HCl to elute metabolite-3, ATA, and metabolite-1, respectively. The eluate was collected in 25-ml. fractions with a fraction collector, and the radioactivity of each fraction was assayed for C^{14} or a mixture of H^3 and C^{14}. The fractions containing each ATA-metabolite were combined and concentrated. These two ATA-metabolites isolated from bean plants were fed to rats which were housed in all-glass metabolism cages. The incorporation of labels into respired CO_2, urine, and feces were examined. The urine samples from this experiment were also chromatographed to determine whether or not the plant metabolites after being fed to rats have been changed. Generally, the paper chromatograms prepared from the urine of rats fed with plant ATA-C^{14} metabolite can be scanned directly with a gas-flow chromatogram scanner, while in those prepared from the urine of rats fed with H^3 and C^{14} ATA-metabolite the activity was too low to permit direct scanning. In this case, the areas on the chromatogram corresponding to ATA, combined plant ATA-metabolites and any new metabolite formed, according to C^{14}-chromatogram, were eluted with water, and the ratio of H^3 to C^{14} of each aqueous extract was measured by a liquid scintillation counter.

Results and Discussion

Relationship of Urinary Excretion of Labeled Metabolite and Oral Dosage of ATA in Rats. A study of 26 adult rats which were given an oral dosage of ATA-5-C^{14} ranging from 1 to 200 mg. per rat, showed that there was no significant difference in the rate of urinary excretion with change in dosage. The average recovery of radioactivity was 85% in the first 24-hour period and 2.1% in the second 24-hour period (Table I). Paper chromatographic separation of these urine samples and direct scanning of the chromatograms for radioactive spots revealed that as the ATA dosage increased, the formation of ATA-metabolite as per cent of dose decreased (Figure 1). The rat appears to have a limited ability to metabolize the ATA molecule. Acetylation of amino groups has been observed in most species of animals, and this reaction may also be taking place in the ATA molecule in rats. However, recrystallization of a mixture of authentic

Table I. Per Cent Recovery of Radioactivity in Urine and Feces of Rats Following a Single Oral Dose of ATA

ATA Dosage, Mg. per Rat	Number of Rats Used	Average Total Radioactivity Found in Urine and Feces, % of Administered Dose: 0 to 24 hours	24 to 48 hours
1	9	86	2.3
5	2	79	2.3
10	2	81	1.7
50	3	89	1.6
100	2	87	2.2
200	3	83	2.1

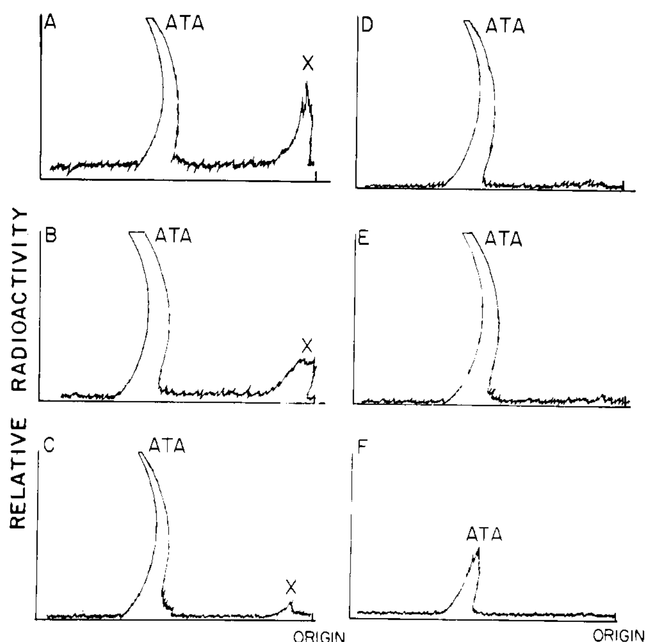

Figure 1. Scanning records of chromatograms from the 24-hour urine sample of rats fed with varying doses of ATA-5-C^{14}; X = rat ATA metabolite

A, 1 mg.; *B,* 5 mg.; *C,* 10 mg.; *D,* 50 mg.; *E,* 100 mg.; *F,* 200 mg.

3-acetamido-1,2,4-triazole prepared according to the method of Fredrick and Gentile (*3*), and ATA-metabolite isolated from rat urine did not give a constant specific activity. In fact, all radioactivity was completely lost after the fourth time of recrystallization in an alcohol and water mixture, providing conclusive evidence that the ATA molecule does not undergo acetylation reaction at amino group in rats, and the metabolite observed in rat urine was not 3-acetamido-1,2,4-triazole.

Accumulation and Rate of Elimination of ATA-5-C^{14} in Male Adults after a Single Oral Dose of 200 Mg. per Rat. In this series, eight male rats were fed ATA-5-C^{14} by stomach tube and sacrificed at a predetermined period of 2 to 48 hours. Various tissues were freezedried, ground, and counted as described previously (*2*). Table II shows the comparative concentration of ATA-5-C^{14} in dried tissues, in which the maximum concentrations were reached between 2 and 6 hours after ATA administration. The radioactivity in all tissues decreased rapidly after 12 hours and reached very low levels in 2 days. Kinetic analyses from the radioactivity accumulation data in various organs indicate that the average half-time for ATA clearance is 4.2 hours (3.4 to 5.1), which is slightly greater than that obtained previously with a 1-mg. dose (*2*). Since there was a great variation in different animals regarding rate of elimination, the slight difference in half-time elimination found for 1 mg. and 200 mg. dosages appears to be insignificant.

Metabolism of ATA-H^3 and ATA-5-C^{14}. Previous work has shown that when ATA-5-C^{14} was fed to rats, the urine contained at least one labeled metabolite. This metabolite can be demonstrated by paper chromatography and isolated quantitatively from urine samples by ion exchange column chromatography. When a mixture of ATA-H^3 and ATA-5-C^{14} was fed to rats, the H^3 to C^{14} ratio of this metabolite was greatly reduced as compared with the original ratio of ATA. From 19 feeding experiments of male and female rats, the average ratio of H^3 to C^{14} in this metabolite was 0.7 to 1 for male rats and 1.4 to 1 for female rats (Table III). The ATA excreted in the urine also showed a reduction in H^3 to C^{14} ratio from the initial mixture. It had an average of 8.3 to 1 in the case of males and 8.9 to 1 in the females as compared with 11 to 1 for the initial ATA mixture. This indicates that the tritium of ATA-H^3 will be subject to exchange reaction in rats with a slightly higher rate for males than for females. Separate experiments were designed to test the stability of tritium atoms of ATA-H^3 in aqeuous solution as a function of pH, and the results indicated that it was very stabile in both basic and neutral pH, with no detectable change in 14 days. However, a 7% loss of tritium activity was noted after 14 days in acidic pH. Therefore, an 18 to 24% loss of tritium activity in ATA molecules after being fed to rats during a 24-hour period was not possible owing to an exchange reaction such as occurred in an aqueous solution, but definitely mediated by a biological system. The age of the rat did not seem to affect the results. The difference in the ratios of H^3 and C^{14} between males and females, as calculated by the t-test, was statistically significant. Since 90% of the tritium activity was in the 5-H position of the ring with the balance amount in the amino hydrogen atoms, an average of 94% loss of tritium activity in the ATA-metabolite from

Table II. Comparative Accumulation of C^{14} in Various Tissues of Male Rats Killed after a Single Oral Dose of 200 Mg. of ATA-5-C^{14}

(Radioactivity was expressed as c.p.m. per 100 mg. dry tissue)

	Body Weight, Grams							
	350	337	405	350	355	342	416	400
	Time after Dosing, Hours							
Tissue or Organ	2	4	6	8	12	18	24	48
Blood	4500	597	390	316	345	45	26	12
Heart	330	474	470	266	398	36	21	13
Kidney	535	832	570	492	697	86	65	24
Lung	989	575	420	327	343	73	50	24
Liver	745	528	440	307	403	136	153	82
Spleen	344	492	452	272	344	65	42	12
Muscle	272	406	294	215	228	21	17	0
Testicles	412	829	730	550	421	43	22	0
Urogenital organ	290	491	607	317	312	39	21	14
Stomach	104410	20050	9710	4200	23900	115	34	0
Intestine	620	824	413	541	671	191	85	19
Brain	191	311	396	335	279	52	21	0

Table III. H^3 to C^{14} Ratio in ATA and ATA-Metabolite from the Urine of Rats Fed a Mixture of ATA-H^3 and 5-C^{14} (H^3/C^{14} Ratio 11 to 1)

No. of ATA	Rat No. 68 F		Rat No. 69 M		Rat No. 70 F		Rat No. 71 M		Rat No. 72 F		Rat No. 73 M	
Dosing	ATA	Metab.	ATA	Metab.	ATA	Metab.	ATA	Metab.	ATA	Metab.	ATA	Metab.
1	9.0	1.7	7.7	0.6	9.1	1.7	8.7	1.2	9.4	1.3	9.1	0.6
2	9.1	2.2	7.3	0.7	8.4	0.9	8.2	1.0				
3	8.7	1.3	7.8	0.5	8.1	0.6	7.3	0.6				
4	9.2	1.7	8.6	0.9	9.1	0.9	8.5	0.8				
5	9.0	1.8	9.7	0.9								

Average H^3 to C^{14} ratio for ATA from male rats 8.3 ± 0.7.
Average H^3 to C^{14} ratio for ATA from female rats 8.9 ± 0.3.
Average H^3 to C^{14} ratio for metabolite from male rats 0.78 ± 0.20.
Average H^3 to C^{14} ratio for metabolite from female rats 1.42 ± 0.46.

male rat urine indicated a substitution of 5-H in the ring and also one of the amino hydrogens, while 88% loss in the ATA-metabolite from female rats indicated that only 5-H in the ring was substituted. A difference in the metabolism of histamine in male and female rats has also been reported (7).

Metabolism of ATA-Metabolites Isolated from Bean Plants in Rats. Table IV presents the excretory patterns of C^{14}-labeled ATA metabolite-1 [the same as compound 1 of Carter and Naylor (1)] and metabolite-3 isolated from bean plants and also a sample of bean extract containing H^3 and C^{14}-labeled ATA metabolites. No C^{14} from either metabolite-1 or metabolite-3 was incorporated into respired CO_2. The pattern of elimination for metabolite-1 and metabolite-3 differed greatly in adult rats. An average of 96% of the administered metabolite-1 was recovered in the first 48-hour period with approximately 90% in the urine. On the contrary, the elimination of metabolite-3 was mainly from feces. The total recoveries in four animals were from 67 to 78% even after as long as 172 hours. After termination of the experiment the animals fed with metabolite -3 were killed, and the tissues were dried and analyzed for C^{14} activity. The result indicated that all the radioactivity not recovered in the urine and feces samples still remained in the tissues. No attempt was made to study the nature of the radioactivity. Paper chromatographic separation of these urine samples from rats fed with metabolite-1 showed that most of the radioactivity was unchanged metabolite-1. The presence of two new radioactive spots and absence of ATA spot indicated that metabolite-1 was metabolized but not converted to ATA in rats. The R_f values of these new metabolites in two solvent systems are shown in Table V. Since the sample of metabolite-3 contained a small amount of ATA, the presence of ATA in rat urine fed with metabolite-3 will not give a definite answer as to whether or not this metabolite may be converted to ATA. Examination of radioautograms prepared from these samples reveals the absence of any new metabolite.

Five observations were made on rats that were fed bean extract prepared from a mixture of ATA-H^3 and ATA-5-C^{14}. The H^3 to C^{14} ratio of this extract was 6.1 to 1 and contained very little free ATA. The average recoverage during a 10-day experimental period was 85% ranging from 79.0 to 98.8%.

This observation also indicated a longer retention time for one of the ATA bean metabolites in rats. Herrett and Bagley (4) reported the isolation of three 3-amino-1,2,4-triazole metabolites in Canada thistle and demonstrated that the third metabolite (unknown III) is herbicidally more active than amitrole. This observation together with the authors' finding of a longer retention time for one of the ATA bean metabolites necessitates a further research on ATA plant metabolites. Measurements of H^3 to C^{14} ratio of 24-hour urine samples showed an average of 5.2 to 1 (4.7 to 5.7) which was reduced from the original extract of 6.1 to 1. Paper chromatographic separation of these urine samples with 2-propanol-ammonia-water solvent and measurement of H^3 to C^{14} ratio from areas representing rat metabolite of ATA (R_f 0.00), bean metabolites of ATA (0.03 to 0.10), and ATA (R_f 0.46), revealed no detectable amount of radioactivity in the region of rat metabolite of ATA, while the average value of the eluate from the bean metabolites region was 3.0 to 1. This low ratio (as compared with 6.1 to 1 for original extract) would indicate that this area may contain another metabolite(s) with low H^3 to C^{14} ratio formed by the rat, and the 5-hydrogen of this metabolite may be substituted as with the ATA molecule. Apparently, the solvent systems used for the separation of plant metabolites from ATA, or rat ATA metabolite from ATA, cannot separate the new rat metabolite(s) from plant ATA metabolites. Attempts are now being made to isolate H^3 and C^{14} labeled ATA bean metabolites separately for rat feeding study.

Table IV. Per Cent Recovery of Radioactivity in Various Fractions Eliminated by Rats Fed C^{14} or C^{14} and H^3 Labeled ATA-Metabolites from Bean Plants

Labeled ATA Metabolites from Beans	Amount Fed, C.P.M. × 10^3	Body Wt., G.	Duration of Experiment, Hours	% Recovery during This Period				Recovery in First 48 Hours
				CO_2	Urine	Feces	Total	
Metabolite-1(C^{14})	2003	F 250	48	nil	88.9	5.4	94.3	94.3
	1347	M 370	48	nil	89.3	8.7	98.0	98.0
	1675	F 224	48	nil	99.1	2.8	101.9	101.9
	1675	M 404	48	nil	84.1	6.0	90.1	90.1
Metabolite-3(C^{14})	2579	M 425	144	nil	23.7	44.2	67.9	67.7
	2579	F 237	144	nil	27.4	39.2	66.6	65.7
	4460	F 224	172	nil	23.2	54.3	77.5	76.1
	4460	M 404	172	nil	12.9	57.3	70.2	67.1
Mixed Metabolites (H^3 × C^{14})	915.4	F 317	240	...	70.5	9.9	80.4	74.3
	915.4	M 374	240	...	77.1	21.7	98.8	88.2
	915.4	F ...[a]	240	...	78.9	6.7	85.6	81.2
	915.4	M ...	240	...	73.0	7.4	80.4	76.1
	458.0	F ...	300	...	63.0	16.0	79.0	71.0

[a] Body weight was not determined.

Table V. R_f Values of ATA, Metabolite-1, and Metabolite-3 from Bean Plants, and Labeled Compounds from the Urine of Rats Fed ATA Bean Metabolites

Compounds	Solvent Systems, R_f	
	Butanol–acetic acid–H_2O (4:1:1.5)	2-Propanol–NH_4OH–H_2O (8:1:1)
ATA	0.48	0.45
Bean metabolite-1	0.16	0.07
Bean metabolite-3	0.23	0.10
Urine of rat fed metabolite-1		
Spot 1	0.13	0.06
Spot 2	0.27	0.11
Spot 3	0.21	0.15
Urine of rat fed metabolite-3		
Spot 1	0.23	0.10
Spot 2	0.48	0.45

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