Metabolism of Gossypol, Biosynthesized from Methyl-^{14}C- and Carboxyl-^{14}C- Labeled Sodium Acetate, in Rat[1]

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ABSTRACT

Stereospecifically labeled radioactive gossypol was biosynthesized by incubating cotton seedlings with either methyl-^{14}C- or carboxyl-^{14}C-labeled sodium acetate. The respective products were purified as gossypol acetic acid. Each radioactive gossypol acetic acid preparation was dissolved in oil and administered by stomach tube to two rats. A negligible amount of radioactivity was found in the expired air of the rats receiving the gossypol biosynthesized from 1-^{14}C-sodium acetate; however, a significant quantity of radioactivity was found in the expired air of rats that received gossypol labeled from 2-^{14}C-acetate. This indicated that the binaphthalene nucleus of the gossypol molecule was not degraded to CO_2 in the rat. A low level of radioactivity was found in the urine of rats administered either gossypol preparation. In each rat there was radioactivity found in all the tissues that were analyzed; however, the major portion of the radioactivity was excreted in the feces.

INTRODUCTION

Gossypol is a polyphenol found in cottonseeds that is toxic to nonruminant animals when consumed in excessive amounts (1). Presently, neither the reason for gossypol toxicity nor the metabolic fate of gossypol is nonruminant animals is understood completely. Lyman, et al., (2) found that, in chicks administered formyl-^{14}C-labeled gossypol, most of the radioactivity was recovered in the feces, with relatively smaller amounts retained in the tissues. In laying hens, formyl-^{14}C-labeled gossypol was excreted rapidly in the feces and urine, and the major portion of the absorbed gossypol was deposited in the eggs (3). When the metabolic fate of formyl-^{14}C-gossypol was studied in the rat, the expired $^{14}CO_2$ increased sharply from rats fed an iron supplemented diet. This suggested that iron may catalyze the decarbonylation of gossypol in the intestine (4).

In gossypol biosynthesized from methyl-^{14}C-acetate, all the nonring carbon atoms are labeled except carbon 12; however, carbon 12 is the only nonring carbon atom labeled in gossypol biosynthesized from carboxyl-^{14}C-acetate (Fig. 1) (5). These respective preparations of radioactive gossypol were used in the present study to determine the degree to which the binaphthalene rings and the nonring carbon atoms of the gossypol molecule are metabolized in the rat.

EXPERIMENTAL PROCEDURES

Radioactive gossypol was biosynthesized by incubating cotton seedlings with either methyl-^{14}C- or carboxyl-^{14}C-labeled sodium acetate, 2-^{14}C- and 1-^{14}C-acetate, respectively (International Chemical and Nuclear Corp., Irvine, Calif.)

The ^{14}C-labeled gossypol was recovered as the dianilino derivative and was purified as gossypol acetic acid according to the method of Smith (6). The purities of the gossypol acetic acid derived from the 2-^{14}C- and the 1-^{14}C-acetate were 99.0 and 99.6%, respectively, and were radiochemically pure (6,7).

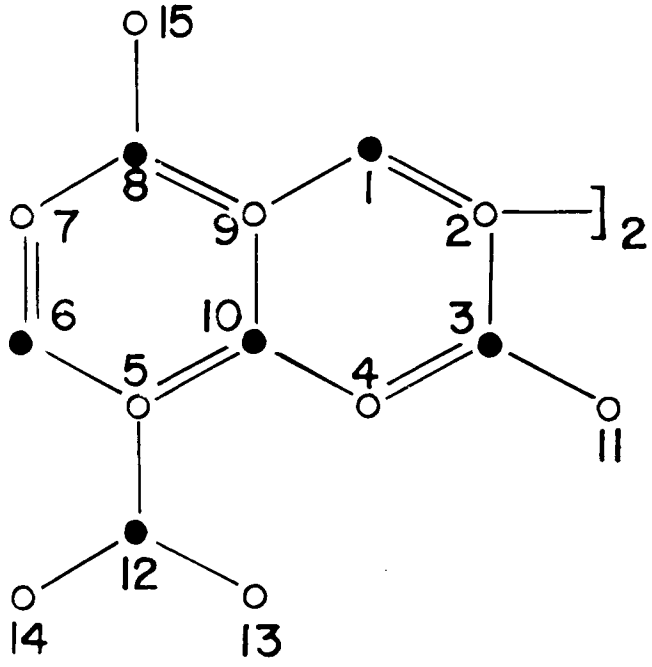

FIG. 1. Indicated utilization of the carbon atoms of acetate in the biosynthesis of gossypol. ○ = Methyl carbon of acetate, and ● = carboxyl carbon of aceate (5).

Gossypol acetic acid (72 mg) was dissolved in 6 ml Wesson oil, and 0.1 ml oil solution was oxidized in a Packard Tri-Carb sample oxidizer (6). The radioactivity in 0.1 ml oil solution of gossypol acetic acid derived from 1-^{14}C-acetate was 292,383 dpm and that from 2-^{14}C-acetate was 344,128 dpm.

Four rats that weighed ca. 150 g were fasted 18 hr prior to the start of the experiment. Each day for 4 days, rats 1 and 2 were intubated orally with 0.5 ml 1-^{14}C-acetate derived gossypol oil solution, except rat 2 received 0.4 ml on day 4. Similarly, rats 3 and 4 were intubated with 0.5 ml 2-^{14}C-acetate derived gossypol oil solution. One of the rats that received 1-^{14}C-acetate derived gossypol was sacrificed on the fifth day, and the other three rats were sacrificed on the eighth day of the experiment.

Each rat was placed in a glass metabolism cage that was designed to control experimental conditions as completely and efficiently as possible. Incoming air first was bubbled through concentrated sulfuric acid to reduce the moisture content of the air and then passed through a column of soda lime to remove the atmospheric carbon dioxide. The expired air was drawn through a primary and then a secondary trap that contained a solution of 1:1 ethanolamine: ethylene glycol monomethyl ether. The feces were collected on a wire screen situated directly below the stage on which the animal rested. The lower portion of the cage tapered into a trough so urinary products passed along the sides of the cage into a trough and down to the urine trap. Water was offered to the rat from a bottle attached to the

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TABLE I

Amount of Radioactivity Recovered from Tissues, Fluids, and Excreta of Rats Orally Administered 1-^{14}C-Acetate Derived Gossypol[a]

Tissue	Rat 1 Specific activity[b] dpm/g	Rat 1 Total activity dpm	Rat 2 Specific activity dpm/g	Rat 2 Total activity dpm
Liver	30,091	189,875	6,363	56,883
Spleen	17,229	8,270	16,247	12,815
Kidney	12,120	15,635	6,988	9,644
Heart	5,328	5,115	4,228	3,340
Lung	6,900	11,040	2,063	2,640
Testes	3,327	5,190	1,333	3,212
Stomach	565,621	565,621	7,790	6,855
Small intestine	207,845	270,198	16,843	32,339
Large intestine	1,038,356	778,767	36,704	44,412
Carcass	2,714	199,756	1,558	171,174
Hide	2,351	50,701	1,219	34,621
Blood	4,444	19,998	722	5,054
Urine	184	8,317	265	16,165
Feces	1,325,936	2,996,615	557,245	4,184,911
CO_2	—	—	—	1,870
Total		5,125,098		4,585,935
Percent recovery		87.6		

[a]Radioactivity administered: rat 1, 5,847,660; rat 2, 5,555,277 dpm.

[b]The specific activity of tissues, stomach, small and large intestines, and feces is expressed as dpm/g dry matter and for blood and urine as dpm/ml. The total radioactivity in the blood represents the radioactivity in the volume of blood collected. The values are the mean of triplicate determinations, except for spleen, heart, and kidneys which were in duplicate.

TABLE II

Amount of Radioactivity Recovered from Tissues, Fluids, and Excreta of Rats Orally Administered 2-^{14}C-Acetate Derived Gossypol[a]

Tissue	Rat 3 Specific activity[b] dpm/g	Rat 3 Total activity dpm	Rat 4 Specific activity dpm/g	Rat 4 Total activity dpm
Liver	11,166	54,267	10,478	59,202
Spleen	8,005	3,282	12,100	5,929
Kidney	10,371	7,260	5,372	6,930
Heart	4,645	3,019	3,583	3,332
Lung	1,903	1,732	1,982	2,735
Stomach	84,254	49,710	40,533	16,213
Small intestine	29,400	38,808	31,060	45,347
Large intestine	60,719	51,611	61,288	77,835
Carcass	1,527	146,020	2,411	237,157
Hide	1,525	46,015	2,521	66,853
Blood	638	4,147	716	4,439
Urine	144	17,280	492	28,044
Feces	811,810	4,408,127	1,073,186	6,084,963
CO_2	—	93,555	—	226,247
Total		4,924,833		6,865,226
Percent recovery		71.6		99.8

[a]The radioactivity administered was 6,882,560 dpm/rat.

[b]The specific activity of stomach, small and large intestines, and feces is expressed as dpm/g dry matter; and for blood and urine, it is expressed as dpm/ml. The total radioactivity in the blood represents the radioactivity in the volume of blood collected. The values are the mean of triplicate determinations, except for spleen, heart, and kidneys which were in duplicate.

roof of the cage, and food was placed in a small compartment directly off the stage.

The rats were anesthetized with diethyl ether, and blood was collected from each by heart puncture. Each rat then was sacrificed, and the hide, the digestive tract, and visceral organs were removed; and all were weighed, including the carcass. Each carcass then was homogenized in a Waring blender. The stomach, small and large intestines, and feces taken from each rat were dried in a vacuum oven at 60 C for 24 hr, weighed, and then ground to a powder in a mortar with a pestle. The contents of the stomach and large intestines were not removed and, thus, contributed to the wt and radioactivity of each organ. Samples of the blood, urine, visceral organs, stomach, large and small intestines, feces, hide, and carcass from each rat were prepared as described for oxidation in a Packard Tri-Carb sample oxidizer (8).

To determine the radioactivity in the expired carbon dioxide of each rat, 2 ml aliquots were taken from each ethanolamine trap and transferred to counting vials that contained 10 ml scintillation liquid. The scintillation liquid contained 4 g 2,5-diphenyloxazole and 0.1 g 1,4-bis-[2-(5-phenyloxazolyl)]-benzene/liter of a 1:1 toluene:ethylene glycol monomethyl ether solution.

All radioactivity measurements were carried out in a Packard Tri-Carb liquid scintillation spectrometer and were corrected for background and quenching. The counting error was less than 5%.

RESULTS AND DISCUSSION

The radioactivity found in the tissues, blood, urine, feces, and expired air of the rats administered 1-^{14}C-acetate derived gossypol and 2-^{14}C-acetate derived gossypol is shown in Tables I and II. Rat 1 was sacrificed 24 hr after receiving the final oral intubation of 1-^{14}C-acetate derived gossypol. Since there was no detectable $^{14}CO_2$ in the expired air of rat 1, rats 2, 3, and 4 were not sacrificed until 3 days later. Four days after the final intubation of 1-^{14}C-acetate derived gossypol, there was only a negligible quantity of $^{14}CO_2$ collected from rat 2. Figure 1 shows that of the 12 carbon atoms labeled in 1-^{14}C-acetate derived gossypol, ten are located in the binaphthalene ring and two are located at position 12. One-sixth of the total radioactivity or 925,881 dpm administered to rat 1 was contributed by carbon 12. Since less than 1/10% of the recovered radioactivity was found in the expired air from rat 2 and none was found from rat 1 (Table III), it is unlikely that the binaphthalene rings or the isopropyl groups are degraded to carbon dioxide. However, there was a significant amount of radioactivity in the expired carbon dioxide of rats 3 and 4 which received the 2-^{14}C-acetate derived gossypol. In 2-^{14}C-acetate derived gossypol, there is a total of 18 ^{14}C-labeled carbon atoms. Of the radioactivity administered to rat 3 and 4, 44% is contributed by the carbon atoms attached to the binaphthalene rings (Fig. 1). The radioactivity found in the expired CO_2 of rats 3 and 4 was 3.1% and 7.4% of the total radioactivity contributed by the substituent carbon atoms. If only the carbonyl groups were converted to CO_2 in rats 3 and 4, the radioactivity in the expired air would be 12.2% and 29.6% of the total radioactivity incorporated into the formyl groups. Since negligible amounts of CO_2 were expired from rats intubated with 1-^{14}C-acetate derived gossypol, it appears that only the for-

TABLE III

Percentage of Recovered Radioactivity Found in Visceral Organs, Gut, Feces, CO_2, Urine, Blood, Carcass, and Hide of Rats Orally Administered 1-^{14}C-Acetate Derived and 2-^{14}C-Acetate Derived Gossypol

Tissue	Rat 1 %	Rat 2 %	Rat 3 %	Rat 4 %
Visceral organs	4.59	1.93	1.41	1.14
Gut	31.50	1.82	2.85	2.03
Carcass and hide	4.89	4.49	3.90	4.43
Blood	0.39	0.11	0.08	0.07
Urine	0.16	0.35	0.35	0.41
Feces	58.47	91.25	89.51	88.63
CO_2	0.00	0.04	1.90	3.30

myl carbon of gossypol is metabolized to CO_2 by the rat. The observation on the formation of CO_2 from the formyl carbon agrees with that of Abou-Donia, et al., (4) who reported that, 4 days after an oral dose of formyl-^{14}C-labeled gossypol to rats, 10.6% of the radioactivity was recovered in the expired air. They also found ca. 2% of the radioactivity in the urine. In the present study, there was a low level of radioactivity in the urine collected from the rats that were intubated with either 1-^{14}C-acetate derived or 2-^{14}C-acetate derived gossypol. Even though the radioactivity in the urine collected from rats 2, 3, and 4 was ca. twice that of rat 1 (Table III), it is doubtful that the urine is a major route for the excretion of gossypol or gossypol metabolites from the body.

The stomach, small intestine, and large intestine of rat 1 contained a high level of radioactivity. Four days after the final intubation of gossypol, radioactivity was found in the stomach of rats 2, 3, and 4. Since the epithelial lining of the stomach is permeable to the lipid-soluble, unionized form of drugs (9), gossypol probably penetrated the wall of the stomach. In the three rats that were sacrificed 3 days after rat 1, the recovered radioactivity in the gut was reduced 30% (Table III). A day after the final intubation of 1-^{14}C-acetate derived gossypol, 50% of the recovered radioactivity was found in the feces. However, ca. 90% of the recovered radioactivity was found in the feces collected from the rats that were sacrificed 3 days later (Table III). Radioactivity was found in the visceral organs, and the amount of the recovered radioactivity in these tissues was reduced in the rats that were sacrificed 3 days after rat 1. Since the radioactivity in the visceral organs and intestines decreased and the radioactivity in the feces increased, this indicated that gossypol or gossypol metabolites were being excreted into the intestine via the bile. It previously has been shown that appreciable quantities of dietary or injected gossypol are found in the bile of swine (10-13).

The results of this study confirm the work of other investigators that, when gossypol is absorbed, it is deposited in the tissues throughout the body (10,11). The radioactivity decreased markedly in most of the tissues from the rats that were sacrificed 3 days after rat 1. This was not true for the carcass and hide. The percentage of the recovered radioactivity from the carcass and hide in rats 2, 3, and 4 was ca. equal to the radioactivity in the carcass and hide of rat 1. Apparently, the gossypol that is deposited in the musculature is not as readily eliminated from the body as the gossypol deposited in the visceral organs.

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