SYNTHESIS OF AMINOGUANIDINE-C^{14}

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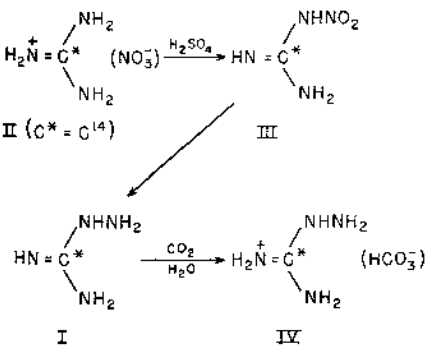
This investigation reports the synthesis of aminoguanidine-C^{14} (I) used as a tracer in studying its physiologic effects in animals. The starting material for the synthesis comprised guanidine-C^{14} nitrate (II) which was converted to nitroguanidine-C^{14} (III) by reaction in sulfuric acid followed by catalytic reduction (Lieber and Smith, 1936) and precipitation as aminoguanidine-C^{14} bicarbonate (IV) by carbonate ion (Thiele, 1898). All intermediate active substances were isolated to serve as references for analysis by paper chromatography and isotope activity. The objective of the synthesis was to prepare IV with a specific activity of approximately 1 μ c/mg.



Experimental

Aminoguanidine-C^{14} bicarbonate (IV). Previously chilled concentrated sulfuric acid, 1.2 ml, was placed in a 3 ml reaction container, kept in an ice-bath for the duration of the reaction. A mixture of 73.0 mg of II and 395 mg of unlabeled guanidine nitrate was added very slowly over 90 minutes to the sulfuric acid with constant stirring, maintaining the temperature below 20°. The mixture was allowed to stand overnight at room temperature followed by addition of 10 g of crushed ice. After one hour, the white precipitate was collected by suction filtration. The solvent was evaporated under high vacuum and precipitation effected by the addition of 10 g of crushed ice giving a small additional amount of III. The combined precipitate was redissolved in 5 ml of boiling water yielding III, by standing overnight, as fine long needles. The product was collected by suction filtration and the mother liquor evaporated to dryness, recrystallization giving additional III. The mother liquor was analysed for activity before every such recovery. A 477 μ g sample of III was retained for analysis, the remaining 320 mg of III being used for the succeeding step. The 320 mg of III was transferred into a 500 ml centrifuge bottle together with 4 ml of fifteen per cent acetic acid, 10 ml of water and 50 mg of platinum oxide catalyst. The hydrogen uptake was stoichiometric after 150 minutes on a mechanical shaker. The catalyst was

filtered off and washed with a few drops of cold water. Sodium bicarbonate, 1.5 g, was added to the combined filtrates and the resulting solution maintained in the refrigerator overnight. The white precipitate of IV was recovered by suction filtration, washed twice with 2 ml of cold water, twice with 2 ml of ethanol, and once with 2 ml of dry ether and finally, air-dried. The resulting white material had a decomposition point of 171-172° similar to that of unlabeled aminoguanidine bicarbonate as prepared by the method of Thiele. The yield was 325 mg of III, 54.1 percent.

Analysis. All the labeled and unlabeled compounds involved were identified for their chemical and isotope purity by paper chromatograph and for specific activity. Both methods gave exact checks for identity. The activity peaks obtained on the radiological chromatograph on the C^{14} compounds corresponded to the spots on the chromatograms when analysed chemically. The chromatograms were scanned at a speed of ¾-inch per minute, at 900 volts and 3,000 counts per minute. Standard was carried out with unlabeled-guanidine nitrate, 1.467 mg; nitroguanidine, 1.055 mg, and; aminoguanidine bicarbonate, 1.759 mg, respectively. Each sample was dissolved in saline solution (1 ml). The three compounds were then spotted on 1½-inch Whatman Paper No. 1 and developed in a chromatography chamber using a mixture of 1-butanol (60 ml), acetic acid (15 ml) and water (25 ml) for development. After the chromatograms were developed and dried, they were dipped in ferricyanide reagent. The *reagent* was prepared from equal volumes of 10 percent potassium ferricyanide and 10 percent sodium hydroxide diluted with nine volumes of water. Before using the reagent was mixed with an equal volume of acetone. Reddish-orange spots were obtained from which the R_f values were calculated. The data obtained are summarized in Table 1. The paper

TABLE 1.—Paper Chromatography on Unlabeled Compounds[a].

Sample	g/ml	Am't Spotted ∂	Am't of Sample γ	R^f x 100 Value
1. Guanidine Nitrate	1.467	10	14.7	50
2. Guanidine Nitrate	1.467	20	29.4	50
3. Nitroguanidine	1.055	10	10.5	57
4. Nitroguanidine	1.055	10	21.0	57
5. Aminoguanidine Bicarbonate	1.759	10	17.6	52
6. Aminoguanidine Bicarbonate	1.759	20	35.2	52

a Results of duplicate independent runs.

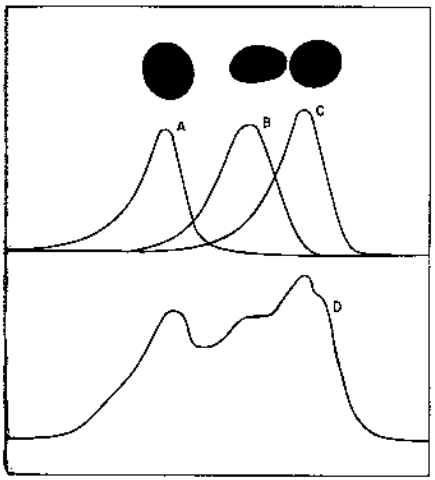


FIG. 1.—Autoradiogram traces and chemical identification of chromatograms: (A), guanidine -C¹⁴ nitrate; (B), nitroguanidine -C¹⁴; (C), aminoguanidine -C¹⁴ bicarbonate; and (D), mixture of all three simultaneously.

chromatography on the C¹⁴-compounds was performed in exactly the same manner, the only difference being that the C¹⁴-labeled chromatograms were first scanned by the chromatograph. Curves with activity peaks were recorded on the locations where the labeled compounds were present. The chromatograms were identified for isotope purity as well as composition against the known unlabeled standard chromatograms. The chromatograms scanned on the Ferro chromatograph showed the location of the particular compound on each strip. One curve was obtained for each substance (Figure No. 1) in order to confirm isotope purity and to mark the location on the chromatogram. The labeled chromatograms were then dipped in ferricyanide reagent. Reddish-orange spots appeared in identical

TABLE 2.—Paper Chromatography on C¹⁴-Labeled Compounds.

Sample	g/ml	Am't Spotted ∂	Am't of Sample γ	R^f x 100 Value
Guanidine C¹⁴—Nitrate	497	10	4.97	50
Guanidine C¹⁴—Nitrate	497	10	4.97	50
Nitroguanidine C¹⁴	477	50	23.85	57
Nitroguanidine C¹⁴	477	50	23.85	57
Aminoguanidine C¹⁴—[a]	577	50	28.85	52
Aminoguanidine C¹⁴—	577	50	28.85	52
Guanidine C¹⁴—Nitrate[b]	497	10	4.97	
Nitroguanidine C¹⁴	477	50	23.85	50-57
Aminoguanidine C¹⁴	577	50	28.85	
Guanidine C¹⁴—Nitrate[b]	497	10	4.97	
Nitroguanidine C¹⁴	477	50	23.85	50-57
Aminoguanidine C¹⁴	577	50	28.85	

[a] Aminoguanidine C¹⁴ was used as the bicarbonate.
[b] A mixture of all three compounds were spotted on one chromatogram simultaneously.

TABLE 3.—Activity Analysis on C^{14}-Labeled Compounds.

Sample	Am't Used ō	Dilution Factor	NCPM	μc/ml	μc/mg	Ave. μc/mg	Ave. μc/mM
Background	0	0	20	0	0	0	0
Background	0	0	20	0	0	0	0
Benzoic Acid C^{14}—Standard	100	1.0	940	0.00111	.....	.....	.....
Benzoic Acid C^{14}—Standard	100	1.0	940	0.00111	.....	.....	.....
Guanidine C^{14}—Nitrate	10	100	32160	4.125	8.032	8.032	1.000
Guanidine C^{14}—Nitrate	10	100	32160	4.125	8.032		
Nitroguanidine C^{14}	20	50	1420	0.906	1.935		
Nitroguanidine C^{14}	20	50	1420	0.903	1.936	1.937	0.200
Nitroguanidine C^{14}	50	20	3600	0.910	1.940		
Nitroguanidine C^{14}	50	20	3580	0.909	1.939		
Aminoguanidine C^{14}—Bicarbonate	20	50	1030	0.675	1.433		
Aminoguanidine C^{14}—Bicarbonate	20	50	1040	0.678	1.435	1.433	0.195
Aminoguanidine C^{14}—Bicarbonate	50	20	2480	0.669	1.430		
Aminoguanidine C^{14}—Bicarbonate	50	20	2640	0.671	1.432		

places where the activity peaks had been previously recorded. Both the spots and the peaks corresponded to the reddish-orange spots obtained in the unlabeled compounds. Table 2 summarizes the R_f values found for the C^{14}-labeled compounds. The *activity analysis* of all three of the C^{14}-labeled compounds are summarized in Table 3. The counts were compared with a benzoic acid C^{14} standard.

ACKNOWLEDGMENT

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SUMMARY

Aminoguanidine-C^{14} bicarbonate has been synthesized with a specific activity of 1.433 microcuries per milligram or 0.195 microcuries per millimole by conversion of guanidine-C^{14} nitrate to nitroguanidine-C^{14} and subsequent reduction to aminoguanidine-C^{14} by catalytic hydrogenation.

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