TABLE II.—RESULTS WITH KNOWN MIXTURES, MG./100 ML.

No.	Sulfacetamide		N^1-Benzoyl sulfanilamide		Sulfathiazole		Total	
	Found	Added	Found	Added	Found	Added	Found	Added
1	0.810	0.800	0.589	0.600	0.637	0.600	2.036	2.000
2	0.404	0.400	1.005	1.000	0.630	0.600	2.039	2.000
3	0.366	0.400	0.812	0.800	0.803	0.800	1.981	2.000
4	1.227	1.200	0.409	0.400	0.368	0.400	2.004	2.000

TABLE III.—ASSAY RESULTS OF OINTMENT

No.	Sulfacetamide, %	N^1-Benzoylsulfanilamide, %	Sulfathiazole, %	Total, %
1	2.765	3.749	3.363	9.877
2	2.947	3.744	3.343	10.034
3	2.799	3.840	3.406	10.045
Average	2.837	3.777	3.371	9.985
Claim	2.860	3.700	3.420	9.980

solvent ether. The suspension was extracted with four 50-ml. portions of 1 *N* hydrochloric acid. The combined acid extracts were washed in a 500-ml. separator with 25 ml. of solvent ether and filtered through a Whatman No. 41 filter paper into a 500-ml. volumetric flask. The filter was washed and the volume made up with 1 *N* hydrochloric acid. A 10-ml. aliquot was pipetted into a 100-ml. volumetric flask and the volume made up with distilled water. A 200-ml. portion of 1 *N* acid was similarly treated to prepare the blank.

The absorbances of the sample solution at 220, 235, and 280 mμ, measured against the blank, were used in the calculations as above to obtain the results in Table III.

CONCLUSION

A spectrophotometric procedure has been developed for rapid and fairly accurate determination of three individual sulfa drugs in combination with each other without prior separation. Though the combination is not the one very frequently used, the procedure could, perhaps, be extended to other combinations as well.

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Synthesis of Methylglyoxal-bis-guanylhydrazone-C^{14}

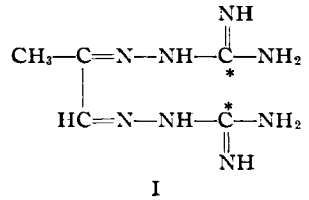
By VINCENT T. OLIVERIO and CHARLENE DENHAM

Methylglyoxal-bis-guanylhydrazone (methyl GAG), an antitumor agent in current animal and clinical trials, has been synthesized with isotopic carbon (C^{14}) in excellent yield and radiochemical purity for pharmacological studies.

NUMEROUS reports have recently appeared relating the inhibitory effects of guanylhydrazones, particularly methylglyoxal - bis - guanylhydrazone (methyl GAG), on various animal and human neoplasms (1–7). To facilitate studies on the metabolic fate and mechanism of action of these agents in man and animals, we have synthesized radioactive methyl GAG-C^{14} (I) in excellent yield and radiochemical purity.

Methyl GAG may be prepared by the reduction of nitroguanidine with zinc dust in acetic acid to aminoguanidine (8), followed by condensation with pyruvaldehyde (9). The aminoguanidine may also be prepared, alternatively, as originally described for pilot plant production (8), by methylation of thiourea with dimethyl sulfate followed by hydrazinolysis of the S-methylisothiuronium sulfate.

For the preparation of methyl GAG-C^{14} on the milligram scale, the latter procedure was chosen because: (*a*) appreciable quantitites of zinc salts were coprecipitated with addition of sodium bicarbonate after reduction of nitroguanidine, even in the presence of ammonium chloride, resulting in an impure product; (*b*) the specific activity (mc./mmole) of the commercially available C^{14}-guanidine nitrate was only one-fourth as high as the less expensive C^{14}-thiourea; and finally; (*c*) the overall reaction yield (30%) of the first procedure was appreciably lower than the yield (70%) of the alternate



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Received April 27, 1962, from the Clinical Pharmacology and Experimental Therapeutics Section, Medicine Branch, National Cancer Institute, Bethesda 14, Md.
Accepted for publication May 25, 1962.
Guanidine-C^{14}, 1,1′-(methylethanedilidenedinitrilo)dihydrochloride monohydrate, is the full chemical name. The abbreviated name, methyl GAG-C^{14}, is employed throughout the text.
The thiourea-C^{14} was obtained from New England Nuclear Corp., Boston, Mass.
Pyruvaldehyde (43%) was obtained through the courtesy of Union Carbide Chemicals Co., New York, N. Y.

method. Thus, in combination with several minor modifications in manipulation and apparatus for adaptation to the semimicro scale, the dihydrochloride salt of methyl GAG-C^{14} was prepared in 99% radiochemical purity with a specific activity of 1.95 mc./mmole.

PROCEDURE

S-Methylisothiuronium-C^{14} Sulfate.—The method was adapted from "Organic Syntheses" (10). In a 20-ml. round bottom flask fitted with a condenser and a soda-lime trap were mixed 10 mmoles of thiourea-C^{14} [50.6 mg. of thiourea-C^{14} (15 mc./mmoles) and 710 mg. of unlabeled thiourea] and 1 ml. of water. To this was added, dropwise, 1 ml. (10 mmoles) of freshly distilled dimethyl sulfate and the mixture was gently refluxed for 1 hour. The reaction mixture was concentrated on a steam bath in a stream of nitrogen to approximately one-half the original volume. Upon cooling, the solidified contents were triturated with several milliliters of ethanol, filtered, and washed with ether. The white solid material was suspended in 10 ml. of a warm mixture of 95% ethanol and ether (1:1), filtered, and washed with ether. The first crop of crystals weighed 406 mg. and melted, with decomposition, at 242°. Concentration of subsequent filtrates to an oil, precipitation with ether, and recrystallization from a mixture of 95% ethanol and either (1:1) yielded an additional 783 mg. of product melting at 242–244°, with decomposition. The total yield was 1.188 Gm. (85%).

Aminoguanidine-C^{14} Bicarbonate.—The procedure was an adaptation of a pilot plant scale reaction in "Organic Syntheses" (8). Hydrazine sulfate (1.095 Gm., 8.4 mmoles) suspended in 3 ml. of water was solubilized by the measured, dropwise, addition of 12 *N* sodium hydroxide solution until neutral to congo red. A second equal volume of sodium hydroxide solution was then added. This hydrazine solution was rapidly added through a reflex condenser to a solution of 1.188 Gm. (4.2 mmoles) of S-methylisothiouronium-C^{14} sulfate and 1 ml. of water in a 20-ml. round-bottom flask. The reaction mixture was stirred magnetically for 1 hour at room temperature. Evaporation of the solution *in vacuo* on a steam bath to one-half the original volume yielded a negligible amount of hydrated sodium sulfate crystals which were removed by filtration. The filtrate was warmed to 30° and glacial acetic acid was added to pH 5. Approximately 1 Gm. of sodium bicarbonate was added to the solution which was stirred at room temperature for 1 hour or until precipitation was complete. The white aminoguanidine bicarbonate salt was filtered, washed with cold water, and dried *in vacuo* at room temperature. The product weighed 1.043 Gm. (90.5%) and melted, with decomposition, at 175°.

Methylglyoxal-bis-guanylhydrazone-C^{14}.—The procedure of Thiele (9) was adapted. A mixture of 1.043 Gm. (7.6 mmoles) of aminoguanidine-C^{14} bicarbonate, 1 ml. of 12 *N* hydrochloric acid, and 0.5 ml. of 95% ethanol was refluxed 30 minutes in a 20-ml. round-bottom flask to give white needles of the hydrochloride salt. To the cooled reaction mixture were added, dropwise, 0.85 ml. (6 mmoles) of 43.2% pyruvaldehyde (partially decolorized by treatment with Darco-G60) and 2 ml. of 95% ethanol. The mixture was stirred 45 minutes at ice-bath temperature. The reaction mixture was poured into 50 ml. of acetone to give a white flocculent precipitate of the bis-guanyldhydrazone dihydrochloride monohydrate. The product was filtered and washed with cold acetone and ether. After drying *in vacuo* at room temperature, the pure compound weighed 982 mg. (3.57 mmoles, 93%) and melted at 256–257°, with decomposition.

The specific activity of the pure radioactive product (1.95 mc./mmoles) was determined by liquid scintillation counting of 0.2-ml. aliquots of a standard aqueous solution (100 mcg./ml.) dissolved in 18 ml. of 30% methanol in toluene containing 0.3% of 2,5-diphenyloxazole and 0.01% of 1,4-bis[2-(5-phenyloxazolyl)]benzene. An absolute counting efficiency of 63% was obtained when samples were counted in plastic bottles. When glass counting bottles were used there was a rapid loss of counts in the samples due to the absorption of the methyl GAG on the surface of the glass container (11).

The radiopurity of the product was assayed by high voltage paper electrophoresis and by paper chromatography in two solvent systems, followed by autoradiography of papers and counting of radioactivity in all spots (12). High voltage electrophoresis (40 v./cm.) of the drug on Whatman No. 3MM paper at pH 3.5 in 0.05 *M* ammonium formate buffer, resulted in a single spot migrating toward the cathode and containing more than 99% of the radioactivity of the load. No radioactive contaminant was detected. In a descending system of *n*-propanol, 1 *N* hydrochloric acid, and water (3:1:1) methyl GAG-C^{14} had an R_f of 0.36 and contained more than 99% of the total load radioactivity. In butanol, 95% ethanol, and water (4:1:1), the compound had an R_f of 0.15 and no radioactive contaminent. In both systems the R_f values for the radioactive compound were identical with those of authentic methyl GAG as located by ferricyanide nitroprusside reagent (13). Lastly, the ultraviolet absorption spectrum at pH 6 of the radioactive methyl GAG was in agreement with authentic methyl GAG.

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