AN EFFICIENT AND CONVENIENT SYNTHESIS OF 5-(3,3-DIMETHYL-^{14}C-1-TRIAZENO)-IMIDAZOLE-4-CARBOXAMIDE

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SUMMARY

Reaction of dimethylamine-^{14}C with a four-fold molar excess of 5-diazoimidazole-4-carboxamide in methanol followed by column chromatography of the product on basic alumina afforded 5-(3,3-dimethyl-^{14}C-1-triazeno)imidazole-4-carboxamide in 70-75% yield and greater than 99.9% radiochemical purity. The preparation is suitable for chemical and biological mechanistic studies.

Key words: 5-(3,3-Dimethyl-^{14}C-1-triazeno)imidazole-4-carboxamide, DTIC, Dacarbazine, 5-diazoimidazole-4-carboxamide.

INTRODUCTION

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, DIC, dacarbazine, NSC-45388), a structural analogue of 5-aminoimidazole-4-carboxamide (AIC), is the most effective single agent in the palliative management of human disseminated malignant melanoma (1). In combination with other drugs it is also clinically useful in the treatment of soft-tissue sarcomas (2) and malignant lym-

phoma (3). DTIC elicits a variety of biologic responses in experimental ani-
mals, including antitumor activity (4,5,6) carcinogenicity (7), and teratogeni-
city (8). The mechanism of action of DTIC, however, remains obscure; it has
been suggested to act both as an alkylating agent (9) and to interfere with
de novo purine synthesis (10). Biochemical studies at both the cellular and
the clinical level have been severely hampered by the limited availability of
DTIC labeled radioisotopically in the side-chain methyl groups. In this report
we describe a convenient microscale synthesis of DTIC-dimethyl-[14]C (DTIC-DM-^{14}C)
(Figure 1) in good yield and excellent radiochemical purity.

FIGURE 1

*Denotes [14]C
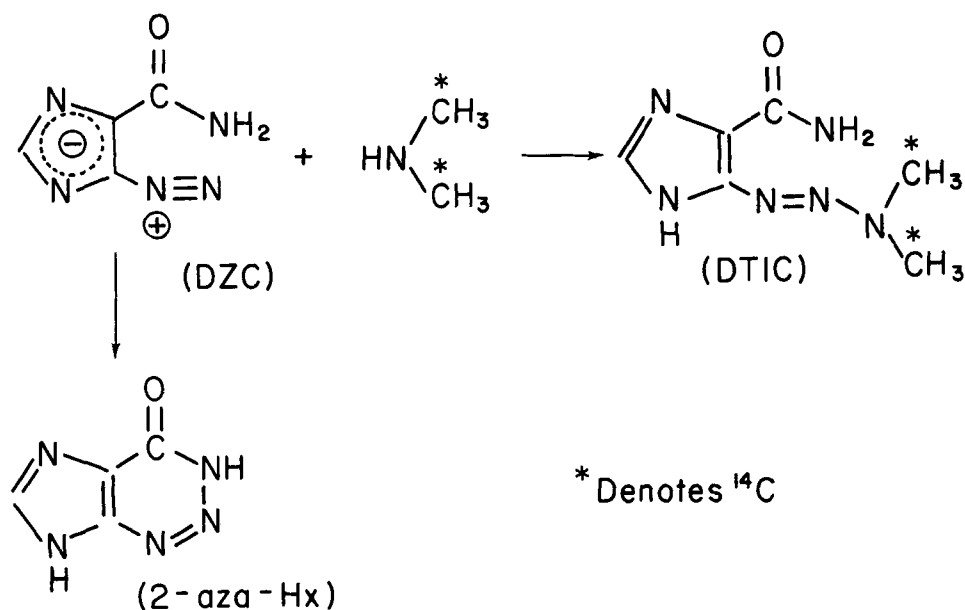
MATERIALS AND METHODS

Dimethylamine-^{14}C hydrochloride (15.15 mCi/mmole) was purchased from Amer-
sham Searle Corporation. 5-Diazoimidazole-4-carboxamide (DZC) was synthesized
from 5-aminoimidazole-4-carboxamide hydrochloride (Sigma Chemical Company)
according to the method of Shealy et al. (11,12). 2-Azahypoxanthine (2-aza-Hx)
was prepared by intramolecular cyclization of DZC (11). DTIC was generously

provided by the Drug Development Branch, Drug Research and Development, Division
of Cancer Treatment, National Cancer Institute. Column chromatography was car-
ried out on Woelm basic aluminum oxide, activity grade I (Alupharm Chemicals,
New Orleans) and thin layer chromatography (tlc) was performed on glass plates
(20 x 5 cm) coated with silica gel (60 F-254), cellulose (300), or aluminium
oxide (F-254) (Brinkmann Instruments, New York). All chromatograms were exam-
ined under short wavelength (254 nm) ultraviolet radiation. Cation-exchange re-
sin (AG 50W-X12) was purchased from Bio-Rad Laboratories, California. Radio-
activity was determined with a Packard model 3375 Tricarb liquid scintillation
spectrometer using "PCS" (Amersham/Searle) counting solution; quenching was
corrected by comparison of channel ratios of external standards. All experimen-
tal procedures were conducted in the dark or under subdued light.

<div align="center">EXPERIMENTAL</div>

5-(3,3-Dimethyl-^{14}C-1-triazeno)imidazole-4-carboxamide

Dimethylamine-^{14}C hydrochloride (5.35 mg, 65.6 μmoles; 15.15 mCi/mmole)
was dissolved in anhydrous methanol (1.0 ml) and the solution was transferred
to a 10 ml round-bottom, one-necked flask equipped with a magnetic stirring bar.
A pellet (ca 100 mg) of sodium hydroxide was added and the flask was immedi-
ately fitted with a distillation assembly. The receiving vessel (10 ml) was
immersed in a dry-ice/acetone bath at -78° and the contents of the flask were
protected from the atmosphere by a drying-tube containing Aquasorb^R (Mallin-
ckrodt). While being stirred rapidly, the solution was gradually heated to 90°
on an oil-bath and the temperature was maintained constant until most of the
methanol had distilled. The temperature was then raised to 120° to remove re-
sidual volatiles. After being cooled to ca. 50°, anhydrous methanol (1.0 ml)
was added and the distillation was repeated. The combined distillate was
allowed to warm to room temperature while protected from atmospheric moisture
and DZC (36.0 mg, 262.2 μmole) was added. The flask was stoppered and the con-

tents were stirred at room temperature in the dark for 3 hr. The resulting

pale-yellow slurry was evaporated to dryness with slow stirring under a gentle

current of dry nitrogen (flow-rate approximately 20 ml/min) at room temperature

using the apparatus shown in Figure 2. Unreacted dimethylamine-^{14}C was trapped

on the strongly-acidic cation-exchange resin[†] while methanol condensed in the

cold-finger.

FIGURE 2
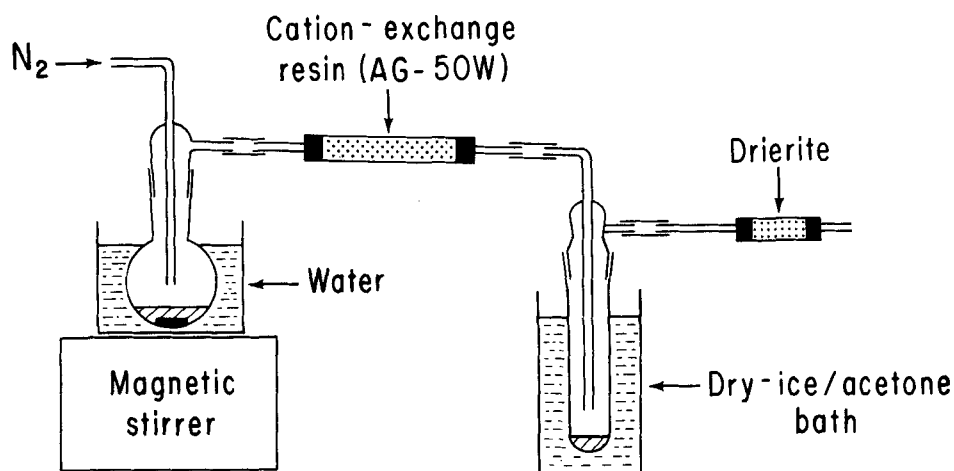
[†] The column was prepared as follows: The resin, suspended in methanol,

was transferred to a glass column (10 x 1 cm) fitted with removable sintered-

glass end-plates. The resin was washed with anhydrous methanol (100 ml), then

the column was drained by applying a back-pressure of dry nitrogen. Finally,

the resin was dried by passing nitrogen through the column at a flow rate of

50 ml/min for 30 min.

The residue was transferred to a 100 ml flask with 3 x 5 ml aliquots of

methanol. A further 25 ml of methanol was added to the suspension and stirring

was continued until a clear solution was obtained. Aluminum oxide (0.75 g) was added and the mixture was evaporated to dryness under reduced pressure at 30°. The residue was suspended in chloroform (3.0 ml) and transferred with the aid of a Pasteur pipette to a column of aluminum oxide (2.3 g; 10 cm x 0.7) made up in the same solvent. To ensure complete transfer of the product, the preadsorption procedure was repeated using a further 0.25 g of aluminum oxide.

DTIC-DM-^{14}C was eluted from the column with chloroform-methanol (3:2); fractions of 5 ml each were collected. The rate of elution was monitored by counting 5 µl aliquots of each fraction and by subjecting 10 µl aliquots to tlc on aluminum oxide. Under uv radiation DTIC-DM-^{14}C appeared as a dark quenching spot at R_f = 0.40. DZC and 2-ara-Hx, used as standards, remained at the base-line.

More than 95% of the radioactivity applied to the column was eluted in the first 30 ml. However, elution was continued until only negligible counts were recovered (the total volume of solution collected was approximately 70 ml). The column was drained, extruded, dried and examined under uv radiation. A dark quench extended about one-third of the length of the exudate confirming that the by-products of the reaction had been retained on the column.

Fractions containing DTIC-DM-^{14}C were combined, filtered, and evaporated to dryness under reduced pressure at 30°. The off-white residue was dried under vacuum over phosphorus pentoxide. The radiochemical purity of the product was determined by tlc. Examination of the chromatograms revealed, in each instance, only a single quenching spot which exhibited chromatographic mobility identical to an authentic sample of DTIC (Table 1). The chromatograms were divided into 1 cm lengths and each section was scraped directly into a counting vial. Water (0.5 ml) was added to elute the products and the radioactivity was determined as described above. Over 99.9% of the counts were located in the quenching areas.

The uv spectra of DTIC-DM-^{14}C in 0.1 $\underline{N}$ HCl and in methanol were identical to those of the unlabeled compound. The specific activity of the product was

15.15 mCi/mmole. The overall yield for the reaction, based on gravimetric,

spectrophotometric, and radiochemical analysis, was 70-75%. The product was

stored in a tightly stoppered vessel in the dark at -20°. Samples preserved in

this manner showed less than 1% decomposition after 3 months. Samples stored

for longer periods could be routinely purified by filtration, in methanolic

solution, through a short column of alumina; this procedure obviates handling

and stability problems associated with recrystallization.

TABLE 1

R_f values of DTIC-DM-^{14}C and analogues[a]

Adsorbent	Solvent[b] System	DTIC-DM-[14]C	DTIC	DZC	2-Aza-Hx
Silica	A	0.35	0.37	0.52	0.35
	B	0.05	0.04	0.43	0.25
Alumina	C	0.40	0.40	0.03	0.00
Cellulose	D	0.53	0.53	--	0.17

[a]Ascending tlc

[b]All proportions v/v

(A) Chloroform - methanol (3:1)   (C) Chloroform - methanol (3:2)

(B) Ethyl acetate - methanol (9:1)   (D) Butanol - conc. ammonia (7:3)

DISCUSSION

The above route to DTIC-DM-^{14}C is based upon Shealy and Krauths' method

(13) for the synthesis of unlabeled DTIC. In the latter procedure, DZC is re-

acted with a large excess of dimethylamine in ethyl acetate; the unreacted amine

does not cause purification problems because it is readily removed by evapora-

tion. The method is obviously unsuitable for the preparation of DTIC-DM-^{14}C since the yield of product, based upon the amount of incorporated amine, is extremely low. To maximally utilize dimethylamine we have used instead a 4-fold molar excess of DZC. The by-products of the reaction are unchanged DZC and 2-aza-Hx, the latter arising via intramolecular cyclization of the former. These compounds, which are not significant contaminants of the unlabeled preparation, are potentially difficult to separate from DTIC. The present synthesis is based on the observation that, unlike DTIC, both DZC and 2-aza-Hx have extremely low chromatographic mobility on basic alumina.

The above procedure possesses several merits. First, the dimethylamine-^{14}C is commercially available as its hydrochloride salt and, following a simple distillation from sodium hydroxide, it is conveniently and efficiently incorporated into DTIC-DM-^{14}C. The yield of DTIC-DM-^{14}C is, in fact, superior to the yield reported for the synthesis of unlabeled DTIC (11). Second, unreacted dimethylamine-^{14}C is trapped on an ion-exchange resin and can be quantitatively recovered by elution with 4 $\underline{N}$ hydrochloric acid; after evaporation, the amine may be stored as the hydrochloride or recycled to produce further DTIC-DM-^{14}C. Finally, the high specific activity and excellent radiochemical purity of the product render it particularly suited for biochemical and biological investigations.

ACKNOWLEDGEMENT

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