DEUTERIUM, TRITIUM, AND CARBON-14 LABELING OF 9-[[2-METHOXY-4-[(METHYLSULFONYL)AMINO]PHENYL]AMINO]-N,5-DIMETHYL-ACRIDINECARBOXAMIDE (CI-921), A NEW ANTITUMOR AGENT

Sheryl J. Hays and C. C. Huang

Warner-Lambert/Parke-Davis Pharmaceutical Research

Ann Arbor, Michigan 48105

SUMMARY

CI-921 2, a potent antitumor agent has been labeled with deuterium and tritium by an exchange reaction employing platinum black as a catalyst. The title compound was carbon-14 labeled from 2-chloro-benzoic-carboxy-^{14}C acid to produce the isethionic salt 2d with a specific activity of 26.9 mCi/mmol. The overall radiochemical yield for the carbon-14 sequence was 22%.

Keywords: Deuterium, Tritium, Carbon-14, CI-921, Antitumor

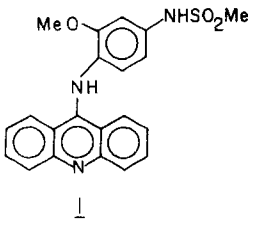
INTRODUCTION

A large number of (acridinylamino)methanesulfonanilide derivatives have recently been investigated for antitumor activity.[1-7] Of these derivatives, amsacrine, N-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulfonamide (1), has been shown to be highly effective against acute leukemia in man and is currently in worldwide clinical use. A new analogue of 1, 9-[[2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino]-N,5-dimethyl-4-acridinecarboxamide (CI-921) (2) is more effective than amsacrine against murine solid tumors, is less myelosuppressive and has greater metabolic stability.[8-11] Preclinical toxicology studies with CI-921 are currently in progress.

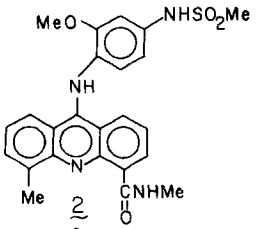
The bioavailability, metabolism and protein-binding studies of 2 required both the tritium and carbon-14 labeled compounds. The deuterium labeling reaction of CI-921 2 was carried out by a catalytic exchange reaction as a preliminary experiment before tritium exchange labeling.

0362-4803/84/060575-12$01.20

Received February 23, 1984



1



2

a. ^{2}H-labeled
b. ^{3}H-labeled
c. ^{14}C-labeled free base
d. ^{14}C-labeled isethionate salt

RESULTS AND DISCUSSION

Deuterium Exchange

The free base of analogue 2 was mixed with acetic acid, deuterated water and platinum black in a sealed vial at 80°C for 18 hours. The deuterium labeled product 2 was analyzed for deuterium incorporation by proton NMR ar mass spectroscopy. Assignment of the aromatic protons in the 300 MHz NMR of the unlabeled compounds was accomplished by decoupling experiments and by comparison of the NMR of the structural fragment of 2, N-(4-amino-3-methoxy-phenyl)methanesulfonamide (3). The aromatic region of the 300 MHz NMR spectra of the unexchanged and deuterium-exchanged product 2a can be seen in Figure 1. An evaluation of the differences in the integration of the two spectra demonstrates that deuterium incorporation is greatest for the protons at the 7, 2, and 6 positions with atom % deuterium of 68%, 68%, and 58% respectively. These positions are the least hindered in the molecule. These results are therefore in agreement with a previous study which suggests that positional deuterium enrichment with a platinum catalyst is a function of the steric rather than the electronic parameters of the substituents.[12] Mass spectral analysis of the deuterium-exchanged compound 2a demonstrates that under the conditions in which the reaction was run, CI-921 2 shows an average incorporation of 2-3 deuterium atoms per molecule.

Proton Assignment	Proton Shift (δ)	Unexchanged Compound (Rel. Integration)	Deuterium-Exchanged Compound (Rel. Integration)	Atom % Deuterium
3	8.59	1.00	0.87	13
1	8.43	1.00	0.97	3
8	8.12	1.00	1.00	0
6	7.93	1.00	0.42	58
2	7.58	1.00	0.32	68
6′	7.52	1.00	0.71	29
7	7.43	1.00	0.32	68
3′,5′	7.06	2.00	1.87	13

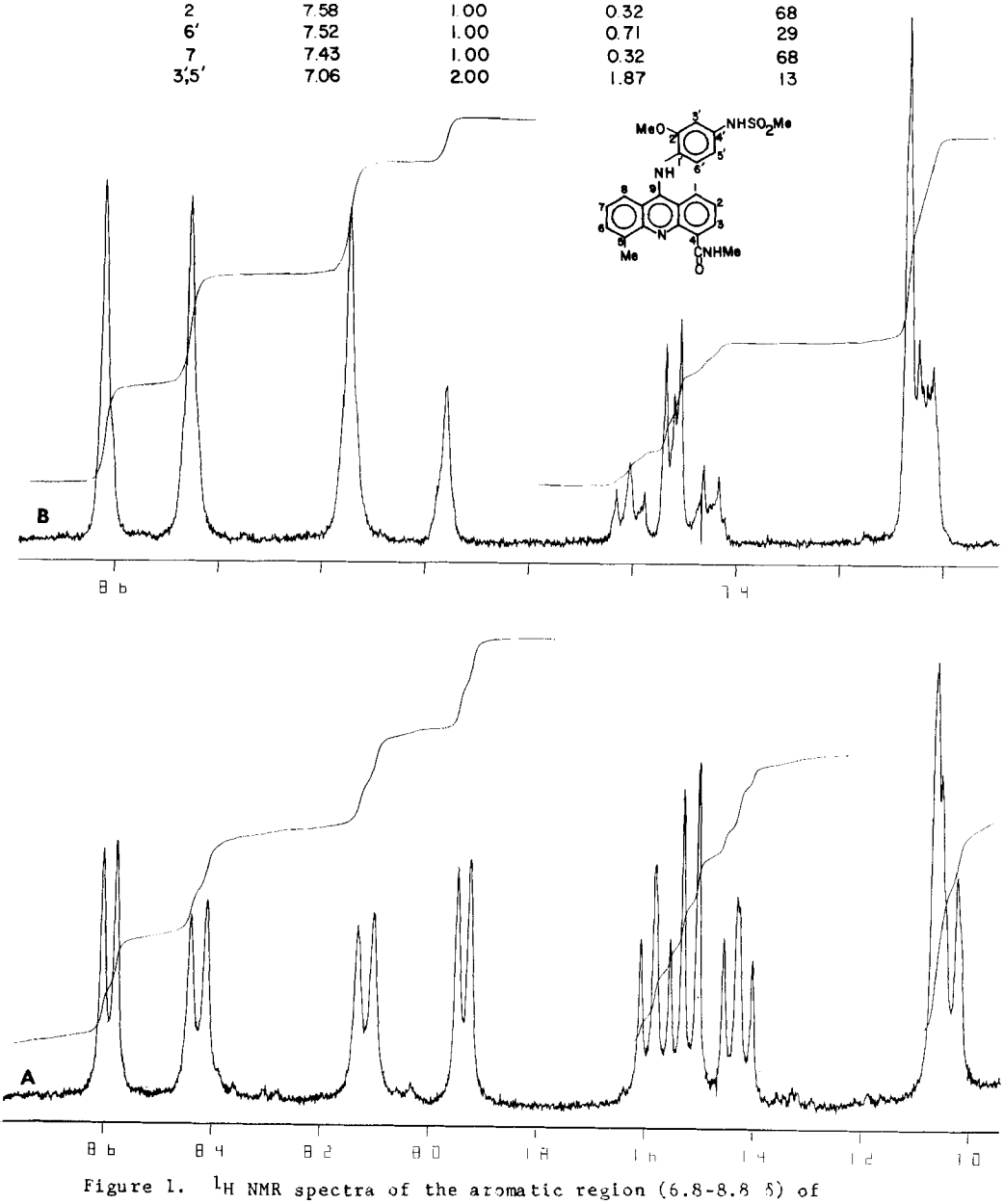


Figure 1. ^1H NMR spectra of the aromatic region (6.8-8.8 δ) of A) Unexchanged CI-921 and B) Deuterium-exchanged CI-921 2a.

Tritium Exchange

The synthesis of generally tritium labeled CI-921 2 was accomplished by a similar catalytic exchange reaction performed by New England Nuclear, using platinum black catalyst and 25 Ci of tritiated water in acetic acid. The product was found to have a specific activity of 4.2 Ci/mmol. The tritium labeled compound 2b was converted to the isethionate salt and diluted to a final specific activity of 59.3 mCi/mmol. The ^{1}H NMR, elemental analysis, melting point, TLC and HPLC were consistent with authentic material. The radiochemical purity was greater than 98%.

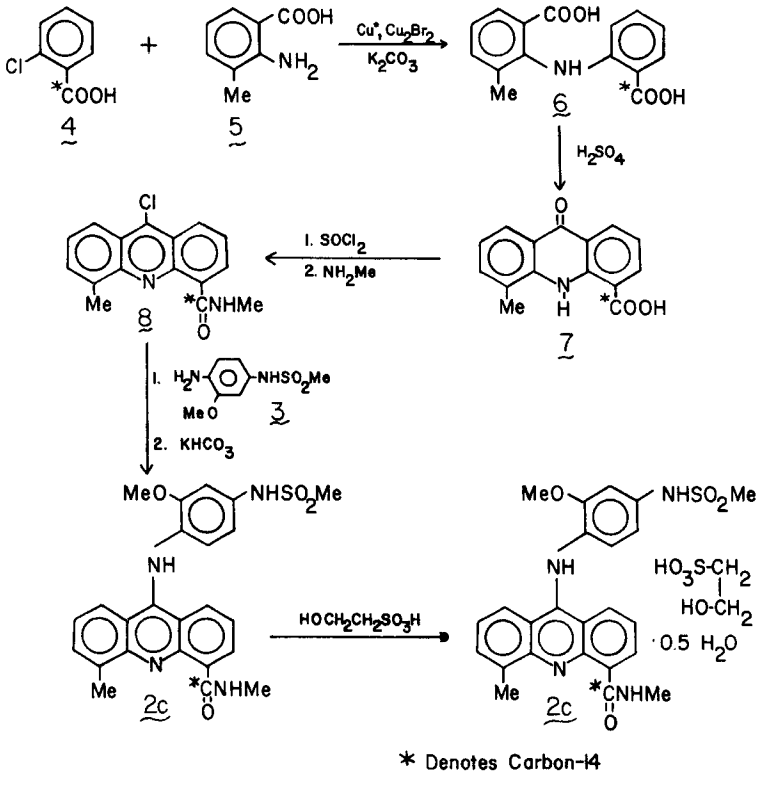
While it is reasonable to assume that positional enrichment of tritium will parallel that for the deuterium experiment, the additional contributions of isotope effects, altered reaction conditions, radiation, and impurities arising from radiolysis can sometimes alter the exchange processes in a quantitative if not qualitative manner.[13] The most notable difference in reaction conditions was the temperatures at which the deuterium and tritium exchange reactions were run (80°C for deuterium versus 25°C for tritium). This in some cases will influence the specificity of labeling.13

Carbon-14 Labeling

The synthesis of the isethionate salt of CI-921-^{14}C 2d was accomplished using 2-chlorobenzoic-carboxy-^{14}C acid (4) as the starting material as shown in Scheme 1. The labeled chlorobenzoic acid 4 was coupled with 2-amino-3-methylbenzoic acid (5) to yield the diacid 6 in a 62% yield. Treatment of 6 with concentrated sulfuric acid at 90°C afforded the yellow acridone 7 in a 83% yield. The acridone 7 was heated with thionyl chloride, followed by treatment with 40% aqueous methylamine to produce the amide 8. Addition of N-(4-amino-3-methoxyphenyl)methanesulfonamide (3) to the amide 8 in refluxing chloroform, yielded the hydrochloride salt which was converted to the free base 2c. The yield from the acridone intermediate 7 was 71%. The free base 2c was converted to the isethionate salt 2d in 60.7% yield to produce the final compound with a specific activity of

26.9 mCi/mmol. The ^{1}H NMR, IR, elemental analysis, melting point, TLC, and HPLC were consistent with authentic material. The radiochemical purity was greater than 99%. The overall radiochemical yield from 2-chlorobenzoic-carboxy-^{14}C acid (5) was 22%.

SCHEME 1



EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Varian XL-300 (300 MHz) spectrophotometer. Chemical shifts were reported in δ (ppm) downfield from tetramethylsilane. Mass spectra were recorded with a Finnigan Series 4000 GC-MS. Liquid scintillation counting was performed with a Packard 574 liquid scintillation counter using Beckman Ready-Solv MP or Mallinckrodt Handifluor liquid scintillation cocktail.

Thin layer chromatography (TLC) was performed on E. Merck silica gel 60 F-254 plates (0.25 mm). The final compound 2 was analyzed using the TLC

systems described as follows: System 1 EtOAc/EtOH/HCOOH (5:5:1) R_f = 0.23; System 2 CH_2Cl_2/MeOH/HCOOH (44:5:1) R_f = 0.14; System 3 2-propanol/H_2O/HCOOH (9:1:.1) R_f = 0.33. Plates were radiochemically analyzed using a Berthold LB 2832 Automatic TLC - Linear Analyzer. High pressure liquid chromatography (HPLC) was performed using a Spectra Physics SP 8700 solvent delivery system, Kratos Spectroflow 773 variable wavelength UV detector, Hewlett-Packard 3390A integrator and United Technologies Packard Tri-Carb RAM 7500 radioactivity monitor.

The tritium exchange reaction was performed by New England Nuclear. 2-Chlorobenzoic-carboxy-^{14}C acid (4) at a specific activity of 25.2 mCi/mmol was purchased from Midwest Research Institute. 2-Amino-3-methylbenzoic acid (5) was purchased from Aldrich Chemical Company. N-(4-Amino-3-methoxyphenyl)methanesulfonamide (3) was synthesized as described previously.[2,3]

9-[[2-Methoxy-4-[(methylsulfonyl)amino]phenyl]amino]-N,5-dimethyl-4-acridinecarboxamide [G-^{2}H] (2a)

A mixture of the free base (46 mg, 0.1 mmol) of 2, 0.3 mL HOAc, 0.2 mL D_2O (99.8 atom %) and 40 mg platinum black were placed in a 0.5 mL V-vial with cap and stirring bar. The solution was stirred in the sealed vial for 18 hours at 80°C. The reaction solution was filtered through a Celite pad to remove the catalyst and the catalyst was washed with MeOH. The solvents were removed under reduced pressure and the resulting acetate salt was dissolved in MeOH and applied to a 2 mm silica gel prep plate eluted with CH_2Cl_2/MeOH/HCOOH (44:5:1). The orange band was eluted from the plate with MeOH and the material was suspended in a solution containing $KHCO_3$ (10 mg), 3 mL H_2O and 1 mL DMF. The solution was heated to 50°C for one hour, cooled to 25°C and filtered. The free base 2a (26 mg) was obtained and analyzed by NMR and mass spectroscopy. ^{1}H NMR of the unexchanged compound (DMSO-d_6 and one drop TFA): 8.59 (d, 1H, arom. 3H, $J_{2,3}$ = 7.7 Hz), 8.43 (d, 1H, arom. 1H, $J_{1,2}$ = 8.8), 8.12 (d, 1H, arom. 8H, $J_{7,8}$ = 8.4 Hz), 7.93 (d, 1H, arom. 6H, $J_{6,7}$ = 6.8 Hz), 7.58 (t, 1H, arom. 2H, $J_{1,2}$ = 8.8 Hz,

$J_{2,3}$ = 7.7 Hz), 7.52 (d, 1H, arom. 6'H, $J_{5',6'}$ = 8.8 Hz), 7.43 (t, 1H, arom. 7H, $J_{6,7}$ = 6.8 Hz, $J_{7,8}$ = 8.4 Hz), 7.06 (s, 1H, arom. 3'H), 7.02 (d, 1H, arom. 5'H, $J_{5',6'}$ = 8.8 Hz), 3.56 (s, 3H, OCH_3), 3.16 (s, 3H, SO_2CH_3), 2.98 (s, 3H, $CONHCH_3$), 2.72 (s, 3H, arom. $-CH_3$).

The NMR of the deuterium exchanged product showed alteration in the splitting patterns and integration in the aromatic region (see Figure 1). Mass spectrum of the unexchanged compound 2, EI (sample mixed with NH_4Cl) m/e 464 (M^+, 24.3), 385 (100). Mass spectrum of the deuterium exchanged compound, EI (sample mixed with NH_4Cl) m/e 469 (7.1), 468 (17.0), 467 (25.7), 466 (22.2), 465 (9.3), 388 (100).

9-[[2-Methoxy-4-[(methylsulfonyl)amino]phenyl]amino]-N,5-dimethyl-4-acridine-carboxamide[G-^{3}H], 2-hydroxyethanesulfonate (1:1), hemihydrate (2b)

A mixture of unlabeled 2 (46 mg, 0.1 mmol), platinum black catalyst (50 mg) in HOAc (0.5 mL) and 25 curies of tritiated H_2O was stirred for 24 hours at 25°C and labile tritium was removed in vacuo using MeOH as a solvent. After filtration from the catalyst, the crude product (826 mCi) was again taken to dryness, in vacuo, and then redissolved in 10 mL of MeOH. TLC analysis of the crude material showed approximately 35% of the desired tritiated compound. A 2.5 mL aliquot (~ 200 mCi) of the methanolic solution was removed. The sample was concentrated and spotted on a preparative silica gel plate (1.0 mm, 20 x 20 cm), which was eluted with EtOAc/EtOH/HCOOH (5:5:1). The orange band corresponding to the product was scraped from the plate and eluted with MeOH. The solvent was removed and the purified material was analyzed for radiochemical purity by TLC. All three solvent systems showed a 5% impurity. The material was dissolved in a minimum of EtOAc/MeOH/HCOOH (5:5:1) and purified by a silica gel column (E. Merck, 230-400 mesh #9385, 1.5 x 15 cm). The orange band was collected, concentrated under reduced pressure and found to contain the formate salt with a specific activity of 4.2 Ci/mmol. Radiochemical purity was shown to be greater than 99%.

The formate salt (12.3 mg) was converted to the free base using 5% $NaHCO_3$ followed by extraction with CH_2Cl_2. Methanolic 2-hydroxyethanesulfonic acid (60 µl, 1.32 M) was added and the solvents were removed. The 2-hydroxyethanesulfonate salt of unlabeled CI-921 (1.0 g) was added to the purified tritiated material and the compound was recrystallized from 2% aqueous EtOH. The red-orange solid (0.52 g) [mp 171-172 (dec), unlabeled authentic sample 171-172 (dec)] had a final specific activity of 59.3 mCi/mmol. An Alltech silica column (10 µ, 4.6 mm ID x 25 cm) was used for HPLC analysis with the following solvent system: CH_2Cl_2/MeOH (4:1) with a flow rate of 1.0 mL/minute. The retention time for the compound was 11.5 minutes employing UV detection at 254 nm. Fractions (0.5 mL) were collected in scintillation vials for radiochemical analysis. Both radiochemical and chemical purity were found to be greater than 98%. An NMR spectrum was taken in DMSO-d_6 and found to be identical to an authentic unlabeled sample. NMR data for the deuterated compound 2a are included in the Experimental.

Anal. calcd for $C_{26}H_{30}N_4S_2O_8 \cdot 0.5H_2O$: C, 52.03; H, 5.17; N, 9.34. Found: C, 52.28; H, 4.94; N, 9.41.

2-[(2-Carboxy-^{14}C-phenyl)amino]-3-methylbenzoic acid (6)

2-Chlorobenzoic-carboxy-^{14}C acid (4) (60.5 mCi, 90% radiochemically pure, 382 mg, 2.4 mmol, specific activity 25.2 mCi/mmol), 2-amino-3-methylbenzoic acid (5) (363 mg, 2.4 mmol) and K_2CO_3 (0.777 g, 5.6 mmol) were mixed with 1-methyl-2-pyrrolidinone (2 mL) and stirred for ten minutes. Copper powder (20 mg)[11] and Cu_2Br_2 (12 mg) were added and the reaction mixture was heated to 150°C over a 35 minute period. The slurry was heated an additional 1.75 hours at 150°C, cooled to 25°C and poured over ice and Celite. The solution was filtered and the solid washed with H_2O. The filtrate was acidified with glacial HOAc and a beige precipitate was filtered and air dried for 18 hours. The diacid 6 was dissolved in 20 mL of hot Na_2CO_3 (5%) and Darco was added to the hot solution and stirred. The solution was filtered through a Celite pad. The still darkened liquid was

mixed with 20 mL of 95% EtOH and glacial HOAc was added dropwise. The solution was cooled to 10°C and the solid (331 mg) was filtered after two hours. The filtrate was concentrated and a second crop of crystals was collected (82 mg). The two crops were combined to yield the diacid 6 (mp 255-256°C, authentic unlabeled sample mp 257-258°C) (37.3 mCi; 413 mg, 61.7% radiochemical yield). TLC on silica gel eluted with EtOAc/EtOH/HCOOH (5:5:.1) indicated that 6 was greater than 95% radiochemically pure.

9,10-Dihydro-5-methyl-9-oxo-4-acridinecarboxylic-^{14}C acid (7)

The diacid 6 (37.3 mCi, 413 mg, 1.5 mmol) was added in small portions using a powder funnel (Kontes #K-299400) to a reaction flask containing 1.0 mL conc. H_2SO_4. The solution was heated to 105°C for 15 minutes and the temperature was lowered to 90°C for 3.25 hours. The reaction was cooled, poured into 5 mL H_2O and stirred. The yellow solid which precipitated was filtered and washed with H_2O. The solid was dissolved in hot DMF/EtOH/H_2O (2:3:5) and conc. NH_4OH. The hot black solution was filtered through a Celite pad and cooled to 10°C for 2.7 days. The yellow solid 7 (31.1 mCi, 317 mg, 83.4% radiochemical yield) (mp 351-353°C, unlabeled authentic sample mp 357°C) was collected and dried in a vacuum oven at 60°C for 30 hours. A TLC of the solid on silica gel eluted with EtOAc/EtOH/HCOOH (5:5:1) indicated that the product was greater than 97% radiochemically pure.

9-[[2-Methoxy-4-[(methylsulfonyl)amino]phenyl]amino]-N,5-dimethyl-4-acridine-carboxamide-^{14}C (2c)

The acridone 7 (31.1 mCi, 317 mg, 1.25 mmol) was mixed with thionyl chloride (2 mL) and DMF (8.0 µl) and the solution was heated to reflux for 0.6 hours. The excess thionyl chloride was removed in vacuo, and final traces were removed by reconcentrating with 5 mL of dry dioxane.

The bright yellow solid was suspended in 4.5 mL of $CHCl_3$ (EtOH free) and cooled to 5°C. Aqueous NH_2CH_3 (40%, 5 mL) was added and the yellow solid dissolved. The reaction was stirred an additional 0.5 hours at 5°C.

The black reaction solution was transferred to a separatory funnel and $CHCl_3$ (20 mL) and H_2O (15 mL) were added to produce two layers. The $CHCl_3$ layer containing the acridyl chloride 8 was washed with H_2O (3 x 10 mL), dried ($MgSO_4$) and filtered. The yellow $CHCl_3$ solution was combined with 1-methyl-2-pyrrolidinone (4 mL), N-(4-amino-3-methoxyphenyl)methane sulfonamide (3)[2,3] (288 mg, 1.33 mmol) and conc. HCl (5 µl). The solution was refluxed for 0.5 hours. A short path distillation head was placed on the reaction vessel and the reaction was refluxed for another hour while the $CHCl_3$ was distilled off. EtOAc (10 mL) was added to the reaction solution and the $CHCl_3$ continued to distill off for an additional hour. The reaction was cooled to 25°C, an additional 10 mL of EtOAc was added and the solution was refrigerated overnight. The red solid was collected by filtration and dried at 60°C in a vacuum oven. A solution of DMF (1 mL), H_2O (6 mL), and $KHCO_3$ (148 mg, 1.48 mmol) was added to the HCl salt and the suspension was stirred at 50°C for five hours. The solution was cooled to 25°C and the orange solid was collected by filtration. The free base 2c (21.9 mCi, 411 mg, 70.9% radiochemical yield) was dried in a vacuum oven at 60°C for 20 hours.

9-[[2-Methoxy-4-[(methylsulfonyl)amino]phenyl]amino]-N,5-dimethyl-4-acridinecarboxamide-^{14}C, 2-hydroxyethanesulfonate (1:1), hemihydrate (2d)

The free base 2c (21.9 mCi, 411 mg, 0.82 mmol) was slurried in DMF (4 mL) for ten minutes at 60-65°C. A methanolic solution of 2-hydroxyethanesulfonic acid (1.32 M, 700 µl, 0.924 mmol) and 4 mL of MeOH were added and the solid dissolved and formed a dark red solution. The solution was heated for an additional ten minutes, filtered through a glass wool plug and cooled to 25°C. EtOAc/hexane (1:1) was added dropwise until the solution turned cloudy. The solution was refrigerated and after 18 hours the 2-hydroxyethanesulfonate salt (0.40 g, 82.3% recovery) was collected by filtration and air dried. The solid was dissolved in 10% aqueous methanol and refrigerated for 2.5 days. The dark orange solid was collected by filtration and dried in a vacuum oven at 70°C for 24 hours to yield the 2-hydroxyethanesulfonate salt

(13.3 mCi, 300 mg, 60.7% radiochemical yield) [mp 268-270°C (dec), unlabeled authentic sample mp 268-270°C (dec)] with a specific activity of 26.9 mCi/mmol.

Thin layer chromatographic analysis was performed on silica gel as described. An Alltech silica column (10 μ, 4.6 mm ID x 25 cm) was used for HPLC analysis with the following solvent system: CH_2Cl_2/MeOH (96:4) with a flow rate of 1.0 mL/minute. The retention time for the compound was 7.5 minutes using sequential monitoring with UV detector at 254 nm and radioactivity detector. Both radiochemical and chemical purity were found to be greater than 99% by TLC and HPLC. NMR and IR spectra were identical to those obtained with an authentic sample. NMR data for the deuterated compound 2a are included in the Experimental. IR (KBr) 1620 (C=O); 1265 (C-O).

Anal. calcd for $C_{26}H_{30}N_4S_2O_8 \cdot 0.5H_2O$: C, 52.03; H, 5.17; N, 9.34. Found: C, 52.17; H, 5.18; N, 9.65.

ACKNOWLEDGMENTS

The authors thank Ms. Susan Uhlendorf, Mr. Bruce Scott, Dr. Gary McClusky, and Dr. Hollis Showalter for their valuable aid and technical assistance.

REFERENCES

1. Atwell, G. J., Cain, B. F., and Seelye, R. N., J. Med. Chem., 15: 611 (1972).
2. Cain, B. F., Seelye, R. N., and Atwell, G. J., J. Med. Chem., 17: 922 (1974).
3. Cain, B. F., Atwell, G. J., and Denny, W. A., J. Med. Chem., 18: 1110 (1975).
4. Cain, B. F. and Atwell, G. J., J. Med. Chem., 19: 1124 (1976).
5. Cain, B. F. and Atwell, G. J., J. Med. Chem., 19: 1409 (1976).
6. Denny, W. A., Cain, B. F., Atwell, G. J., Hansch, C., Panthananicka A., Leo, A., J. Med. Chem., 25: 276 (1982).

7. Denny, W. A., Atwell, G. J., and Baguley, B. C., J. Med. Chem., 26: 1619 (1983).

8. Baguley, B. C., Atwell, G. J., Denny, W. A., Ferguson, L. R., Finlay, G. J., and Wilson, W. R., Abstracts of Papers, 13th International Congress of Chemotherapy, Vienna, Austria, August 28 - September 2, 1983.

9. Denny, W. A., Atwell, G. J., and Baguley, B. C., J. Med. Chem., 27: 000 (1984) in press.

10. Latham, B., Von Hoff, D. D., and Elslager, E. F., Abstracts of Papers, Fourth NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Belgium, December 14-17, 1983.

11. Leopold, W. R., Shillis, J. L., Corbett, T. H., and Jackson, R. C., Proc. Amer. Assoc. Cancer Res., 25: 000 (1984) in press.

12. Fraser, R. R. and Renaud, R. N., J. Amer. Chem. Soc., 88: 365 (1966).

13. Evans, E. A., "Tritium and its Compounds," 2nd edition, John Wiley and Sons, New York (1974).

14. Blatt, A. H., Org. Syn. Coll. Vol. II: 445 (1943).