at 75–80° for 0.5 hr. The reaction mixture was decomposed with water and the separated solid was crystallized from benzene: yield 0.22 g (25%).

(c) The butanol **2** when heated under reflux with EtOH–HCl (4%) gave **3** in 15% yield.

***trans*-2,2-Dimethyl-3-phenyl-4-*p*-(β-pyrrolidinoethoxyphenyl)-7-methoxychroman (34).** (a) Alkylation of **3** with β-pyrrolidinoethyl chloride hydrochloride as mentioned above for **7** gave **34** in 92% yield which was converted to its HCl salt and crystallized from ethanol–ether: mp 164°. Anal. ($C_{30}H_{36}ClNO_3$) C, H, N.

(b) BuLi (35 ml of 19.85%) in THF was added to a suspension of **33** (23.6 g, 0.05 mol) in 250 ml of anhydrous Me_2SO at room temperature under nitrogen atmosphere under good stirring. The resulting red solution was stirred for further 3 hr and then decomposed with 50 ml of cold water. The reaction mixture was then poured over 1.5 l. of an ice–water mixture and extracted with ether. The organic layer was washed well with water and dried (Na_2SO_4), the solvent distilled off, and the residue converted to its HCl salt and crystallized from ethanol–ether: yield 24.8 g (97.4%); mp 164°.

***cis*-2,2-Diethyl-3-phenyl-4-(*p*-hydroxyphenyl)-7-methoxychroman (35).** High-pressure hydrogenation of **24** following the procedure used for **7** yielded **35** in 68% yield. The solid was recrystallized from benzene–hexane: mp 186°. Anal. ($C_{26}H_{28}O_3$) C, H.

***cis*-2,2-Diethyl-3-phenyl-4-*p*-(β-pyrrolidinoethoxy)-phenyl-7-methoxychroman (36).** Alkylation of **35** with β-pyrrolidinoethyl chloride hydrochloride as mentioned above for **33** gave a 93% yield of **36** as a solid compound which was recrystallized from benzene–hexane: mp 133–133.5°. Anal. ($C_{32}H_{39}NO_3$) C, H, N.

***trans*-2,2-Diethyl-3-phenyl-4-*p*-(β-pyrrolidinoethoxy)-phenyl-7-methoxychroman (37).** Isomerization of **36** using BuLi in Me_2SO as mentioned for **34** yielded 81.3% of **37** which was converted to its HCl salt and crystallized from 2-propanol: mp 227°. Anal. ($C_{32}H_{40}ClNO_3$) C, H, N.

Acknowledgment. We are thankful to Dr. Jim Bolger for his help in the reduction of chromene **14** and its subsequent isomerization which greatly facilitated the preparation of **34**.

References and Notes

(1) Communication No. 2049 from the Central Drug Research Institute, Lucknow, India.
(2) (a) H. P. S. Chawla, P. K. Grover, N. Anand, V. P. Kamboj, and A. B. Kar, *J. Med. Chem.*, **13**, 54 (1970); (b) S. Ray, P. K. Grover, N. Anand, V. P. Kamboj, and A. B. Kar, *Indian J. Exp. Biol.*, **9**, 103 (1971); (c) S. Ray, P. K. Grover, and N. Anand, *Indian J. Chem.*, **8**, 961 (1970); (d) *ibid.*, **9**, 728 (1971).
(3) (a) R. W. J. Carney, M. Hill, and W. L. Bencze, U.S. Patent 3340276 (Sept 5, 1967); (b) R. W. J. Carney, W. L. Bencze, J. Wojtkunski, A. A. Renzi, L. Dorfman, and G. DeStevens, *J. Med. Chem.*, **9**, 516–520 (1966).
(4) S. Ray, P. K. Grover, and N. Anand, *Indian J. Chem.*, **9**, 619 (1971).
(5) C. E. Cook, R. C. Corley, and M. E. Wall, *J. Org. Chem.*, **30**, 4114 (1965).
(6) S. Ray, P. K. Grover, and N. Anand, unpublished work.
(7) H. D. Lauson, C. G. Heller, J. B. Golden, and E. L. Severinghaus, *Endocrinology*, **24**, 35 (1939).
(8) E. Allen and E. A. Doisy, *J. Am. Med. Assoc.*, **81**, 819 (1923).
(9) C. W. Emmens, *Methods Horm. Res.*, **2**, 59 (1962).
(10) D. Lednicer, S. C. Lyster, and G. W. Duncan, *J. Med. Chem.*, **10**, 78 (1967).
(11) P. L. Kole, S. Ray, and N. Anand, unpublished work.

Relationship of Molecular Structure to in Vivo Scintigraphic Distribution Patterns of Carbon-11 Labeled Compounds. 3. [11C]Hydantoins

Meldrum B. Winstead,* Shelley J. Parr, Michael J. Rogal, Paul S. Brockman, Ronald Lubcher,

Bucknell University, Lewisburg, Pennsylvania 17837

Archie Khentigan, Tz-Hong Lin, James F. Lamb, and H. Saul Winchell

Medi-Physics, Inc., Emeryville, California 94608. *Received July 14, 1975*

The preparation and γ-scintigraphic evaluation of the in vivo distribution patterns in dogs of a series of structurally related hydantoins labeled with the positron emitting, 20.4 min half-life radionuclide, carbon-11 are described. Carbon-11 labeled HCN was collected in water or aqueous Me_2SO containing carrier KCN following cyclotron bombardment of 99% N_2–1% H_2 gas mixture with 22 MeV protons for 1 hr at 25–35 μA. Five ^{11}C-labeled 5,5-dialkylhydantoins, three [^{11}C]diarylhydantoins, five [^{11}C]-5-alkyl-5-phenylhydantoins, and five [^{11}C]spirohydantoins were prepared by heating, generally under pressure, a mixture of ^{11}C-labeled KCN, which was produced by isotopic exchange with carrier KCN, the corresponding aldehyde or ketone, and excess $(NH_4)_2CO_3$ in aqueous ethanol or Me_2SO solvent. The ^{11}C-labeled hydantoins were dissolved in 1–1.5% aqueous NaOH for intravenous administration to dogs. Total synthesis time was 70–106 min and 1–59 mCi of final product was available for conducting serial in vivo imaging for up to 2 hr or more with the γ-scintillation camera. Carbon-11 activity from all compounds showed initial blood-pool distribution with variable concentration of activity in the brain, lungs, liver, and kidney. All of the ^{11}C-labeled diarylhydantoins, and most having one phenyl substituent, and one having a hexamethylene moiety showed initial accumulation of ^{11}C activity in brain. Carbon-11 labeled 5,5-diphenylhydantoin (dilantin) showed the greatest qualitative accumulation of activity in the brain. Those ^{11}C-labeled hydantoins having a carboxyl substituent showed prominent renal concentration and urinary excretion of activity. Most ^{11}C-labeled hydantoins showed a progressive homogenous whole body distribution of activity in all cellular tissues of the body. The relatively uniform distribution of activity in cellular tissues and slow excretion from the body support the thesis that the pharmacologic action of the hydantoins is related to physical effects on biomembranes rather than to specific chemical interactions with cell constituents.

Data presently available from experiments using ^{14}C-labeled compounds are insufficient to predict adequately the relationship between molecular structure of an organic compound and its in vivo distribution pattern as studied by scintigraphic techniques. This is so for several reasons. The inherent low specific activity of ^{14}C-labeled compounds makes ^{14}C an unsuitable label for evaluation of in vivo distribution patterns of molecules whose in vivo distribution demonstrates a strong "carrier" effect in the range of the quantity of ^{14}C-labeled material

required for the experiment. Studies of in vivo distribution patterns of ^{14}C-labeled compounds require serial sacrifice of essentially identical isogenic animals and assessment of changes in ^{14}C activity in organs in vitro as a function of time after administration. This demands a priori insight in choosing the proper time intervals for such sacrifice and selection of samples of the proper tissues for in vitro assay to detect in vivo distribution behavior of interest occurring at a given time in a given tissue. Such insight is rare except in the study of metabolic behavior which is already well known and in which distribution kinetic studies offer little additional insight. The complexity of proper tissue sampling for assessment of in vivo distribution patterns using ^{14}C-labeled compounds cannot be underestimated. The relative accumulation of the ^{14}C label in various portions of a presumed homogeneous tissue may be quite disparate. For example, ubiquitous skeletal muscle, bone, and bone marrow may show wide variations in accumulation of a given substrate in samples of the tissue obtained from different sites in the body, often depending on their relative blood perfusion at the time of the study in the given animal being studied. Comparison of relative ^{14}C activity in the rectus femorus muscle, the femur, and femoral bone marrow with activity in brain for a given compound obtained with ^{14}C in rats may provide limited insight into the contribution of activity in skull and temporalis muscle in imaging the brain of human subjects when a congener of the compound in question is synthesized incorporating a γ-emitting radionuclide. Moreover, the limited in vivo distribution data using ^{14}C-labeled compounds presently in the literature were obtained in a fashion which often provides little insight into prediction of distribution patterns of use in nuclear medical studies involving qualitative and quantitative scintigraphic image interpretation of in vivo radioisotope distribution in man. The methods involved in study of in vivo distribution patterns using ^{14}C as a tracer are not only not applicable in man but also are usually of limited usefulness in any large mammal where large colonies of identical isogenic subjects are unavailable.

We believe that definition of the relationship between molecular structure and the in vivo distribution patterns of organic compounds would have significant implications in nuclear medicine (e.g., in "designing" radiopharmaceuticals to achieve a desired result). We further believe that it would be extremely difficult to obtain such information using ^{14}C as a tracer in a manner which could lead directly to its application in qualitative and quantitative scintigraphic studies in man because of the reasons noted above. Definition of the relationship of molecular structure to scintigraphically determined in vivo distribution can be achieved using ^{11}C as a tracer for organic compounds as long as the in vivo behavior being studied can be defined within the time limits set by physical decay of ^{11}C, which, at present, averages approximately 2 hr. The remarkable usefulness of scintigraphic images in rapidly conveying complex data to the human mind is well established in nuclear medicine. The use of ^{11}C allows for direct imaging and quantitation of tissue–distribution patterns in a fashion which cannot be readily matched by the indirect techniques afforded by the use of ^{14}C. Similarly, use of ^{11}C-labeled agents allows for performance of noninvasive pharmacokinetic studies where one is interested in obtaining data concerning uptake and release of label from a radioactive compound in specific organs and tissues of a single subject.

We have instituted a program of systematic development of techniques for rapid synthesis of families of ^{11}C-labeled compounds and for serial scintigraphic evaluation of their in vivo distribution patterns. The organic synthesis methods used are modifications and time–yield optimizations of known synthetic reactions. The purposes of this effort are (a) to develop methods for making large numbers of ^{11}C-labeled compounds available for present and future study and (b) to survey the in vivo distribution patterns of the compounds synthetized using nondestructive in vivo methodology most applicable to their potential use in nuclear medicine (e.g., γ scintigraphy in intact animals).

In our program of systematic synthesis of ^{11}C-labeled compounds and evaluation of their in vivo distribution by means of γ scintigraphy, we have at present studied a variety of ^{11}C-labeled carboxylic acids, hydantoins, nitriles, amines, cyanate and hydroxyurea, α-amino acids, α-aminonitriles, cyanohydrins, and α-hydroxy acids. The first paper in this series described our results with 26 ^{11}C-labeled carboxylic acids[1] and the second with 22 ^{11}C-labeled α-aminonitriles.[2] The present paper describes our results with 18 ^{11}C-labeled hydantoins.

Experimental Section

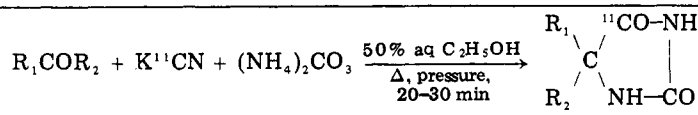
Materials and Methods. Carbon-11 labeled HCN was produced by cyclotron bombardment of a 99% N_2–1% H_2 gas target with 22 MeV protons using procedures and equipment described previously.[3] The gas stream containing carrier-free [^{11}C]HCN was passed through 25 ml of H_2O or aqueous Me_2SO containing approximately 5 mg each of $(NH_4)_2CO_3$ and KCN. This resulted in ^{11}C labeling of the KCN by isotopic exchange. An average of 761 mCi of [^{11}C]cyanide for 19 runs was trapped in the solution following 1 hr of bombardment at 25–35 μA. Carrier KCN (generally 5 mmol), an equivalent amount of the corresponding aldehyde or ketone, and a 2–10 mmol excess of $(NH_4)_2CO_3$ were added to the solution. Ethanol was added to make a 50–60% aqueous ethanol solution for those reactions not conducted in aqueous Me_2SO. The solution was heated, generally in a pressure reaction apparatus, for 20–60 min and then poured into cold dilute NaOH which dissolved the hydantoin in the basic solution which was subsequently extracted with ether. The alkaline solution was cooled and acidified with concentrated HCl, and the precipitated hydantoin was filtered and washed with cold aqueous alcohol or water. The hydantoin was dissolved in a small quantity of 1–1.5% aqueous NaOH which was administered intravenously to dogs. Typically, the total synthesis time varied from 70 to 106 min, and activity averaging 20 mCi of final product was available for intravenous injection. A weighed sample of the [^{11}C]hydantoin was purified either by recrystallization from aqueous C_2H_5OH or by dissolving in base, extracting with ether, and acidifying the alkaline solution. The precipitated [^{11}C]-hydantoin was dried and specific activity measurements were determined.

In vivo distribution patterns of the intravenously administered ^{11}C-labeled hydantoins were evaluated scintigraphically using a Searle Radiographics HP scintillation camera fitted with a pinhole collimator containing a 1-cm diameter hyperbolic aperature. Such distribution patterns only reflect qualitative to semiquantitative distribution of the ^{11}C label in the body because of variable geometric and absorption factors. True quantitative scintigraphic data and images of ^{11}C distribution in vivo can be obtained using coincidence imaging techniques. Such techniques were not utilized in the present work. Quantitative estimates of organic and tissue distribution of activity were obtained by placing the organs and tissues obtained at necropsy at fixed geometrical positions in front of a thallium-activated NaI scintillation detector and analyzing detected scintillation events using routine methods.

Results

A. Synthesis. The ^{11}C-labeled hydantoins which were prepared in this study are listed in Tables I–IV. The more water-soluble [^{11}C]hydantoins listed in Table I, e.g., 5-(2-methylthioethyl)hydantoin, 5,5-diethylhydantoin, and levulinic acid hydantoin, were isolated by evaporation of the aqueous ethanol reaction mixture to a volume of 5–10 ml followed by cooling and acidification with concentrated

Table I. Preparation of Carbon-11 Labeled 5,5-Dialkylhydantoins



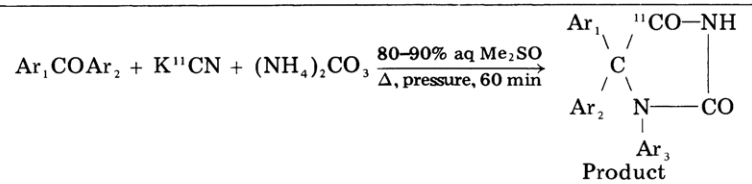
$K^{11}CN$, mCi, t_0 (mmol rxn scale)	R_1	R_2	Product mCi t_0	Product mCi t_{inj}	Radiochemical yield, %	Chemical yield, %	Total synthesis time, min
634 of $Na^{11}CN$ (12)	$CH_3SCH_2CH_2$	H	42[a]	7	7		71
449 (5)	C_2H_5	C_2H_5	163[b]	15	36	50	71
373 (10)	n-C_3H_7	n-C_3H_7	44[c]	2	12		90
327 (10)	i-C_4H_9	i-C_4H_9	22[d]	1	7	11	75
377 (5)	CH_3	CH_2CH_2COOH	111[e]	10	30	15	70

[a] E. Pierson, M. Giella, and M. Tishler, *J. Am. Chem. Soc.*, **70**, 1450 (1948), obtained a 79% yield of methionine hydantoin on a 0.25-mol reaction scale after heating 3-methylthiopropanal (0.25 mol), NaCN (0.5 mol), and $(NH_4)_2CO_3$ (1.17 mol) 4 hr at 50–55° in 50% aqueous ethanol: mp 103–105°. Similarly, D. O. Holland and J. H. C. Nayler, *J. Chem. Soc.*, 3403 (1952), obtained an 89% yield of crude methionine hydantoin by including triethylamine and boiling for 15 min, followed by evaporation to 1–2 ml, and acidification: mp 108°, after recrystallization from water. We have obtained 40–50% yields of methionine hydantoin of mp 98–100° on 6-, 12-, and 25-mmol reaction scales of nonradioactive NaCN and $CH_3SCH_2CH_2CHO$ and a twofold excess of $(NH_4)_2CO_3$ following this latter procedure. The infrared spectrum of product agreed with Sadtler Standard Grating Spectrum No. 13821. Radioactive procedures were conducted on 12-mmol reaction scales in 50 ml of 50% aqueous ethanol in a pressure bottle heated in a boiling water bath for 25 min. [b] S. D. Upham and O. C. Dermer, *J. Org. Chem.*, **22**, 799 (1957), obtained a 64% yield of 5,5-diethylhydantoin on a 1-mol reaction scale after heating 3-pentanone (1 mol), NaCN (1.3 mol), and $(NH_4)_2CO_3$ (3.3 mol) for 10–20 hr at 58–60° in 50–65% aqueous ethanol: mp 163°. In nonradioactive experiments, we obtained 70% averaged yields of 5,5-diethylhydantoin on a 5-mmol reaction scale after heating KCN (5 mmol), 3-pentanone (5.2 mmol), and $(NH_4)_2CO_3$ (20–42 mmol) in 25 ml of 50% aqueous ethanol under pressure for 20 min at 190–200°. The solution was evaporated to a volume of 5 ml prior to acidification with concentrated HCl: mp 165.5–166°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 21186. [c] Errera, *Gazz. Chim. Ital.*, **26** (I), 207 (1890), reported mp 199° for 5,5-dipropylhydantoin as prepared from dipropylcyanoacetamide and Br_2 and KOH. In nonradioactive experiments, we obtained a 65% yield of 5,5-dipropylhydantoin on a 10-mmol reaction scale after heating KCN (11 mmol), 4-heptanone (10 mmol), and $(NH_4)_2CO_3$ (40 mmol) for 30 min at 70° and 15 min at 80° in 25 ml of 60% aqueous ethanol, followed by evaporation to a volume of 10 ml, addition of 5% aqueous NaOH, extraction with ether, and acidification with concentrated HCl: mp 200–201°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 21196. [d] S. D. Upham and O. C. Dermer, see footnote *b*, prepared 5,5-diisobutylhydantoin on a 1-mol reaction scale in 12% yield by heating diisobutyl ketone (1 mol), KCN (1.3 mol), and $(NH_4)_2CO_3$ (3.3 mol) in 50–65% aqueous ethanol at 58–60° for 10–20 hr: mp 147–148°. A. Lumiere and F. Perrin, *Bull. Soc. Chim. Fr.*, **35**, 1022 (1924), reported mp 220° (as prepared from diisobutylcyanoacetamide and alkaline sodium hypochlorite). In nonradioactive experiments, we obtained 5,5-diisobutylhydantoin in averaged yields of 21% on a 10-mmol reaction scale of KCN and diisobutyl ketone and 50 mmol of $(NH_4)_2CO_3$ by heating at 100–200° for 30–60 min in a pressure bottle in 25 ml of 50% aqueous ethanol, followed by evaporation to a volume of 10 ml prior to the addition of 10% aqueous NaOH, extraction with ether to remove unreacted ketone, and acidification with concentrated HCl: mp 219–221°. [e] H. R. Henze and R. J. Speer, ADI Document 1603, supplement to *J. Am. Chem. Soc.*, 64, 522 (1942), obtained levulinic acid hydantoin on a 20-mmol reaction scale after heating levulinic acid (20 mmol), KCN (40 mmol), and $(NH_4)_2CO_3$ (80 mmol) for 2 hr at 60° in 50% aqueous ethanol: mp 156.5–157.5°. K. Pfister et al., *ibid.*, 77, 697 (1955), obtained this hydantoin in 87% yield upon heating levulinic acid (4.3 mol), NaCN (66.5 mol), and $(NH_4)_2CO_3$ (4 lb) in water at 58–60° for 18 hr: mp 159–160°. In nonradioactive experiments, we obtained averaged yields of 46 and 67% of levulinic acid hydantoin on a 5- and 10-mmol reaction scale, respectively, of KCN and levulinic acid and a five- to eightfold excess of $(NH_4)_2CO_3$ by heating at 160–200° for 20–30 min in a pressure tube in 24–30 ml of 25–50% aqueous ethanol, followed by evaporation to a volume of 5 ml prior to acidification with concentrated HCl: mp 154–156°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 22649. The ^{11}C-labeled hydantoin was dissolved in 7.5% aqueous $NaHCO_3$ prior to its intravenous administration to dog.

HCl. 5,5-Dipropyl- and 5,5-diisobutylhydantoin were isolated by evaporation of the reaction mixture to a volume of 10 ml with subsequent cooling and addition of 5 ml of 10% aqueous NaOH, followed by extraction with ether to remove unreacted ketone. The hydantoin was isolated from the alkaline solution upon acidification with concentrated HCl. The ^{11}C-labeled hydantoins listed in Tables II–IV were isolated from the aqueous Me_2SO reaction mixture by the addition of 50–75 ml of 5% aqueous NaOH (which diluted the Me_2SO solvent and converted the hydantoin into the water-soluble sodium salt), followed by extraction with ether to remove unreacted ketone. The crystalline hydantoin was obtained upon subsequent acidification of the alkaline solution with concentrated HCl. The purity and structure of the hydantoin were confirmed by the agreement of melting point and infrared spectrogram data obtained for the product with the respective data reported in the literature. Prior to the ^{11}C reaction, each hydantoin was prepared by reacting the appropriate aldehyde or ketone with nonradioactive KCN and $(NH_4)_2CO_3$ under experimental conditions designed to optimize the yield at minimal reaction times as described in Tables I–IV. The resulting hydantoins were isolated and purified, and their melting points and infrared spectra were determined as a confirmation of their respective data reported in the literature.

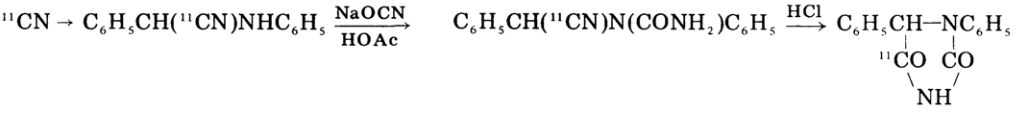
B. In Vivo Distribution. Figure 1 scintigraphically demonstrates the initial in vivo distribution pattern seen during intravenous administration of ^{11}C-labeled hydantoins containing approximately 2 mmol of carrier hydantoin. The particular compound whose results are shown in Figure 1 was ^{11}C-labeled 5,5-pentamethylenehydantoin. In all cases, ^{11}C activity appeared in the veinous drainage of the injection site with variable retention in the vein wall proximal to the site of injection. In several cases, some retention of activity was seen in the region of the valves in the veinous drainage system. In all cases, some initial retention of activity in the lungs was seen. The degree of such retention was variable. As activity cleared the lungs, concentration in liver was seen

Table II. Preparation of Carbon-11 Labeled Diarylhydantoins



K^{11}CN, mCi, t_0 (mmol rxn scale)	Ar_1	Ar_2	Ar_3	Product mCi t_0	Product mCi t_{inj}	Radiochemical yield, %	Total synthesis time, min
159 (15)	C_6H_5	C_6H_5	H	58[a]	2	36	106
2103 (3)	p-ClC_6H_4	p-ClC_6H_4	H	239[b]	7	11	105
640 (5)	C_6H_5	H	C_6H_5	112[c]	1	18	105

[a] H. R. Henze, U.S. Patent 2 409 754 (Oct 22, 1964) [*Chem. Abstr.*, **41**, 1250*d* (1947)], obtained a 67% yield of 5,5-diphenylhydantoin (dilantin) on a 55-mmol reaction scale by heating benzophenone, KCN, and $(NH_4)_2CO_3$ in 60% ethanol for 90 hr. Similarly, in protic solvent systems, e.g., ethylene glycol and trimethylene glycol, dioxane, and ethanolamine, a 97.5% yield of dilantin was obtained after 6 hr at 110° on a 0.5-mol reaction scale: mp 293–296°. In nonradioactive experiments, we obtained a 35–40% yield of dilantin on a 15-mmol reaction scale by heating KCN (18 mmol), benzophenone (15 mmol), and $(NH_4)_2CO_3$ (50 mmol) in 30 ml of 90% aqueous Me_2SO under pressure at 135–140° for 1 hr: mp 296–298°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 5470 and 8026. [b] H. R. Henze and A. F. Isbell, *J. Am. Chem. Soc.*, **76**, 4152 (1954), prepared 5,5-bis(*p*-chlorophenyl)hydantoin in 94% yield on a 0.1-mol reaction scale by heating 4,4′-dichlorobenzophenone (0.1 mol), KCN (0.11 mol), and $(NH_4)_2CO_3$ (0.3 mol) in fused acetamide under pressure at 110° for 9–48 hr: mp 319–320° (recrystallized from dioxane). In nonradioactive experiments, we have prepared this hydantoin in 62% yield on a 5-mmol reaction scale of KCN and the ketone and 42 mmol of $(NH_4)_2CO_3$ upon heating the reagents in 30 ml of 80% aqueous Me_2SO at 190–200° for 45 min under pressure: mp 305–306.5°. In 50% aqueous Me_2SO, the hydantoin was produced in 25% yield: mp 305–306° (unrecrystallized). [c] Carbon-11 labeled 1,5-diphenylhydantoin was prepared by reacting sodium cyanate (4 mmol) with [^{11}C]-α-anilinophenylacetonitrile in 5 ml of acetic acid solvent at 85° for 18 min, with subsequent conversion of the intermediate [^{11}C]-α-ureidonitrile to the [^{11}C]hydantoin upon boiling with 10 ml of concentrated HCl. Chemical yields of nonradioactive optimization experiments were 50–55% (27–35% overall from benzilideneaniline): mp 202–204° [reported mp 204–206°: E. Testa and R. Ettorne, *Arch. Pharm.*, (*Weinheim, Ger.*), **290**, 532 (1957)]. [^{11}C]-α-Anilinophenylacetonitrile was prepared by reacting ^{11}C NaCN (5 mmol) and benzilideneaniline (5 mmol) in 65 ml of 60% aqueous ethanol containing 0.4 ml of acetic acid at 85° for 10 min with subsequent cooling. Chemical yields of nonradioactive optimization experiments were 55–63%; mp 84–85° [reported mp 85–86°: L. Neelakantan and W. H. Hartung, *J. Org. Chem.*, **24**, 1943 (1959)], and 91° [J. S. Sandhu, P. S. Sethi, and S. Mohan, *J. Indian Chem. Soc.*, **48**, 89 (1971)].



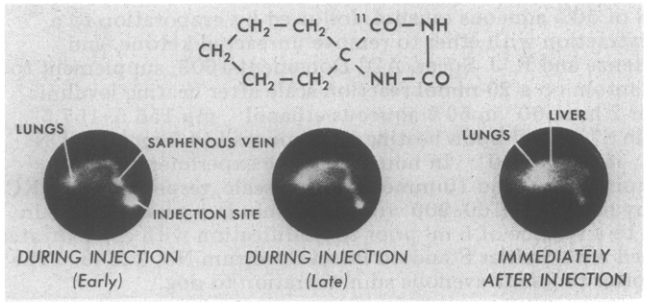


Figure 1. Serial in vivo distribution patterns of ^{11}C activity during and immediately following intravenous administration of ^{11}C-labeled 5,5-pentamethylenehydantoin in 1.2% aqueous NaOH to a dog. Note retention of activity in the vein wall proximal to the site of injection and in the region of the valves in the veinous drainage system. Note initial retention of activity in the lungs and subsequent concentration in the liver.

to a certain extent in all cases, and, in a few cases, concentration of activity was noted in the region of the brain. In most cases, activity was found dispersed throughout the entire body after initial equilibration was complete. In a few cases, concentration of activity in kidneys and excretion in urine was a prominent feature of the in vivo distribution pattern. Further details are noted below.

All members of the ^{11}C-labeled 5,5-dialkylhydantoin series listed in Table I showed the pattern of initial retention of activity in lungs and liver followed by distribution of activity diffusely throughout the body and progressive concentration in kidney and excretion in urine. Levulinic acid hydantoin showed more prominent retention of activity in the vein wall proximal to the site of injection and marked initial retention in lungs, liver, and kidneys and more prompt excretion of activity in the urine than did the other compounds in this group. Left lateral whole body scintiphotos obtained approximately 5 and 30 min after intravenous injection of [^{11}C]levulinic acid hydantoin are shown in the first row of figures in Figure 2. None of the members of this series of 5,5-dialkylhydantoins showed concentration of activity in the brain or significant excretion in the bile.

The in vivo distribution pattern noted with the members of the series of ^{11}C-labeled diarylhydantoins listed in Table II was similar to the pattern noted for the 5,5-dialkylhydantoins, except that all showed some evidence of concentration of activity in the brain, some showed evidence of biliary excretion of activity, and all showed less evidence of renal concentration and urinary excretion of activity in comparison with the dialkyl compounds. Concentration of activity in the brain was noted to a variable degree with most members of this series of compounds but was most marked with ^{11}C-labeled 5,5-diphenylhydantoin (dilantin). As can be seen from the scintigraphs in Figure 3, activity is noted diffusely throughout the brain, and within the first 40 min after administration of [^{11}C]dilantin little activity is noted in the cerebrospinal fluid. Quantitation of activity at necropsy 40 min after administration of dilantin confirmed the concentration of activity in the brain over that in blood. Similar concentrations of activity were observed in kidneys,

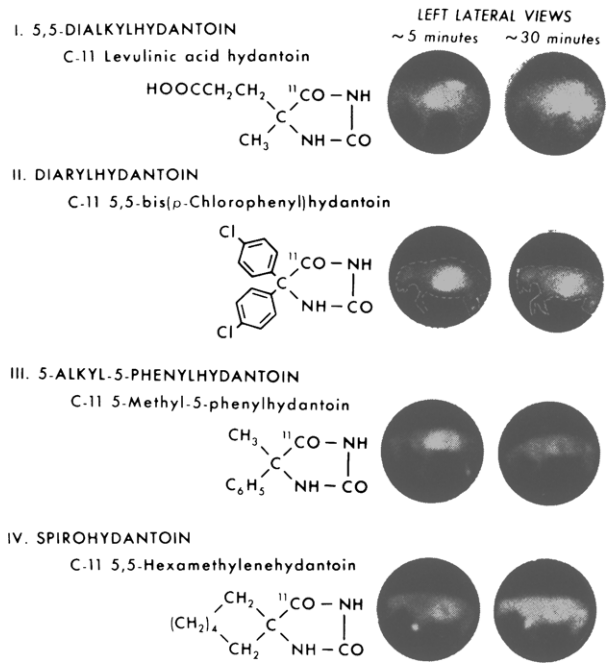


Figure 2. Early and late whole body in vivo distribution of ^{11}C activity following intravenous administration of a representative member of each category of a series of ^{11}C-labeled hydantoins in 1–1.5% aqueous NaOH to dogs. Note the prominent retention of ^{11}C activity in the vein wall proximal to the site of injection and marked initial retention of activity in lungs, liver, and kidneys and prompt excretion of activity in urine observed with [^{11}C]levulinic acid hydantoin. Note that this basic in vivo distribution pattern for the 5,5-dialkylhydantoin category is seen for the remaining three categories of ^{11}C-labeled hydantoins. Note some concentration of activity in the brain with ^{11}C-labeled 5,5-bis(*p*-chlorophenyl)hydantoin. Note the typical initial concentration of activity in lungs and liver followed by diffuse whole body distribution of activity observed with ^{11}C-labeled 5-methyl-5-phenylhydantoin and 5,5-hexamethylenehydantoin. Note some concentration of activity in the brain with the latter compound.

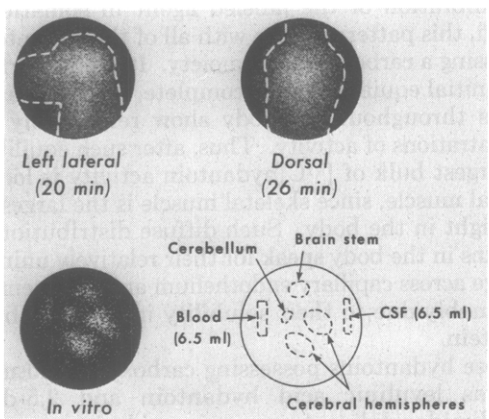


Figure 3. Concentration of ^{11}C activity in the brain following administration of ^{11}C-labeled 5,5-diphenylhydantoin (dilantin) in 1.5% aqueous NaOH to a dog. Note the distribution of activity throughout the brain and the in vitro confirmation of such diffuse accumulation in the brain.

heart and skeletal muscle, lungs, stomach, and pancreas. The highest concentration was noted in the liver. It is noteworthy that activity in skeletal muscle was greater than that in mesenteric fat and omentum, two relatively well perfused lipid storage depots. Left lateral whole body scintiphotos obtained approximately 5 and 30 min after intravenous injection of [^{11}C]-5,5-bis(*p*-chlorophenyl)-hydantoin are shown in the second row of images in Figure 2.

The basic in vivo distribution pattern noted above was again seen with the members of the series of ^{11}C-labeled 5-alkyl-5-phenylhydantoins listed in Table III. Generally, these compounds showed diffuse whole body distribution. Notably, ^{11}C-labeled 2,5-dioxo-4-phenylimidazolidine-4-propionic acid was rapidly concentrated in the kidneys and excreted in the urine. Significant urinary excretion of activity was also noted at 1 hr after administration of ^{11}C-labeled 5-phenylhydantoin. Following administration of ^{11}C-labeled 5-ethyl-5-phenylhydantoin (nirvanol) and 5-phenyl-5-propylhydantoin, there was scintigraphic evidence of concentration of activity in the brain. None of the compounds showed significant early excretion of activity in the bile. The typical initial concentration of activity in lungs and liver followed by diffuse whole body distribution of activity noted with compounds in this series is exemplified by scintiphotos obtained approximately 5 and 30 min after administration of ^{11}C-labeled 5-methyl-5-phenylhydantoin shown in row three of Figure 2. Table V shows the relative concentration of ^{11}C activity in tissues 73 and 80 min after administration of [^{11}C]-5-ethyl-5-phenylhydantoin and [^{11}C]-5-phenylhydantoin, respectively. It is clear that ^{11}C activity is diffusely distributed throughout the body at this time, except for some slight concentration in liver and kidneys. It is noteworthy that 73 min after administration of [^{11}C]-nirvanol ^{11}C activity in the cerebrospinal fluid (CSF) is higher than that in any tissue or in urine and bile.

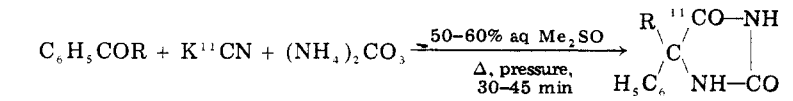
The typical in vivo distribution pattern for hydantoins, in general noted above, was also seen with the series of structurally related ^{11}C-labeled spirohydantoins listed in Table IV. Some concentration of activity in the brain was noted with ^{11}C-labeled 5,5-hexamethylenehydantoin (see scintiphotos in the fourth row of Figure 2). However, initial concentration of activity in the brain was not marked with other compounds in this category. Following initial equilibration, ^{11}C activity was generally diffusely distributed throughout the body with little tissue localization except for modest concentration of activity in the liver.

Discussion

The initial distribution pattern of intravenously administered hydantoins presented in this communication can largely be explained by considering the limited solubility of the compounds in aqueous solution at body pH. Following their administration, the hydantoins injected intravenously in 1.0–1.5% aqueous NaOH tend to precipitate from solution as they are diluted in the neutral aqueous media of the plasma. Such precipitation manifests itself largely as retention of activity along the vein wall proximal to the site of injection and initial retention of activity in the lungs, presumably due to trapping of ^{11}C-labeled precipitate particles having an effective diameter greater than that of the internal diameter of the capillary wall. Initial retention of activity in liver may be related to trapping of colloidal particle-size precipitates which have escaped retention in the pulmonary capillary bed.

The distribution pattern of activity in other tissues appears to be largely a function of blood perfusion rate and relative rates of passage across capillary endothelium and cell membranes. The brain has an unusually high perfusion rate, but because of the selectivity of the capillary endothelium in the brain, most water-soluble materials show poor initial concentration in the brain. It is antic-

Table III. Preparation of Carbon-11 Labeled 5-Alkyl-5-phenylhydantoins



$K^{11}CN$, mCi, t_0 (5 mmol rxn scale)	R	Product mCi t_0	Product mCi t_{inj}	Radiochemical yield, %	Chemical yield, %	Total synthesis time, min
1053[a]	H	347[b]	21	33		83
1084	CH_3	654[c]	59	60	59	71
375	CH_3CH_2	173[d]	14	46	37	74
1618	$CH_3CH_2CH_2$	756[e]	56	47	49	77
491[f]	CH_2CH_2COOH	119[g]	10	24	61	75

[a] This was a 10-mmol reaction scale preparation. [b] H. T. Bucherer and V. A. Lieb, *J. Prakt. Chem.*, **141**, 5 (1934), obtained an 80% yield of 5-phenylhydantoin on a 1-mol reaction scale by heating benzaldehyde, KCN, $NaHSO_3$, and $(NH_4)_2CO_3$ in 50% aqueous ethanol for 5 hr: mp 183–184°. In nonradioactive experiments, we obtained 50–60% yields of 5-phenylhydantoin on a 5-mmol reaction scale after heating KCN (5 mmol), benzaldehyde (7 mmol), and $(NH_4)_2CO_3$ (25 mmol) in 50 ml of 50% aqueous Me_2SO at 70–75° for 25 min and 85–90° for 10 min: mp 182–183°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 24827. [c] H. T. Bucherer and V. A. Lieb, *ibid.*, **141**, 5 (1934), obtained a 90% yield of 5-methyl-5-phenylhydantoin on a 1-mol reaction scale by heating acetophenone, KCN, and $(NH_4)_2CO_3$ in 50% aqueous ethanol for 5 hr: mp 198.5°. In nonradioactive experiments, we obtained 62% yield of this hydantoin on a 5-mmol reaction scale of KCN and acetophenone and 42 mmol of $(NH_4)_2CO_3$ after heating the reagents in 30 ml of 60% aqueous Me_2SO at 175–182° under pressure for 40 min: mp 198–199°. The infrared spectrum of product agreed with Sadtler Standard Prism Spectrum No. 8421. [d] H. T. Bucherer and V. A. Lieb, *ibid.*, **141**, 5 (1934), reported an 85% yield of 5-ethyl-5-phenylhydantoin (nirvanol) on a 1-mol reaction scale by heating propiophenone, KCN, and $(NH_4)_2CO_3$ in 60% ethanol for 6 hr: mp 201–202°. In nonradioactive experiments, we obtained 50% averaged yields of nirvanol upon heating KCN (5 mmol), propiophenone (5 mmol), and $(NH_4)_2CO_3$ (50 mmol) in 30 ml of 50% aqueous Me_2SO at 160–170° under pressure for 45 min: mp 199.5–201.5°. The experimental infrared spectrum agreed with that reported by T. H. Elliott and P. N. Natarajan, *J. Pharm. Pharmacol.*, **19**, 209 (1967). [e] T. J. Thompson, H. L. Bedell, and G. M. Buffett, *J. Am. Chem. Soc.*, **47**, 874 (1925), prepared 5-phenyl-5-propylhydantoin in 75% yield from α-cyano-α-phenylvaleramide and alkaline sodium hypobromite: mp 165–166°. In nonradioactive experiments, we obtained 5-phenyl-5-propylhydantoin in yields of 55% on a 5-mmol reaction scale of KCN and butyrophenone and 42 mmol of $(NH_4)_2CO_3$ after heating the reactants under pressure in 30 ml of 60% aqueous Me_2SO at 180–190° for 40 min: mp 168–169°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 21194. [f] The reaction solvent was 50% aqueous ethanol. [g] M. B. Winstead, F. R. Scholer, and K. H. Wildrick, *J. Med. Chem.*, **9**, 142 (1966), obtained 2,5-dioxo-4-phenylimidazolidine-4-propionic acid in 80% yield from 3-benzoylpropanoic acid (94 mmol), KCN (154 mmol), and $(NH_4)_2CO_3$ (658 mmol) by heating 22 hr in 60% aqueous ethanol: mp 216–217.5°. In nonradioactive experiments, we obtained this hydantoin in 66% yield upon heating KCN (5 mmol), 3-benzoylpropanoic acid (5.5 mmol), and $(NH_4)_2CO_3$ (42 mmol) for 30 min at 190–200° under pressure in 30 ml of 50% aqueous ethanol. The solution was evaporated to a volume of 10 ml, made basic with 10% aqueous NaOH, extracted with ether to remove unreacted 3-benzoylpropanoic acid, and acidified with concentrated HCl, and the product was recrystallized from 70% aqueous ethanol: mp 216°. The experimental infrared spectrum matched Sadtler Standard Prism Spectrum No. 8767. The ^{11}C-labeled hydantoin was dissolved in 7.5% aqueous $NaHCO_3$ prior to its intravenous administration to dog.

ipated that any agent which shows high "blood–brain barrier" permeability would show rapid initial concentration in the brain due to the high perfusion rate per unit mass of brain. Thus, initial rapid accumulation of an agent in brain following its intravenous administration is largely a measure of its "blood–brain barrier" permeability. In the case of the hydantoins, those compounds having one or more phenyl groups (or, in the case of the spirohydantoins, 5,5-hexamethylenehydantoin) showed the greatest tendency toward initial accumulation in brain. Such permeability appears to be due to the solubility in cell membrane lipoprotein imparted to the molecule by the phenyl or hexamethylene groups. Initial equilibration within the brain substance is not accompanied by rapid initial accumulation in free cerebrospinal fluid. With time, the label in the brain slowly diffuses into the CSF and back into the blood. The kinetics of such processes is such that, at times greater than 1 hr after administration, the concentration of activity in CSF is greater than that in brain or blood (the activity in blood having fallen because of loss of label to other extravascular sites).

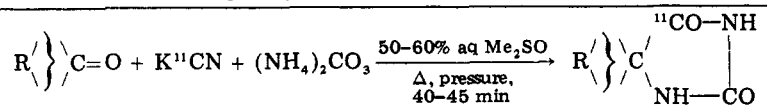
In general, viscera have a much higher blood perfusion rate per unit mass than do somatic tissues such as resting muscle. However, the cellular mass of somatic tissue is much greater than that of viscera. Thus, as noted above, if transcapillary and transmembrane passage of an agent are rapid, one anticipates that following intravenous administration of the agent to an animal at rest there would be initial relative concentration of activity in highly perfused viscera followed by progressive redistribution and reequilibration of the labeled agent in somatic tissue. Indeed, this pattern is seen with all of the hydantoins not possessing a carboxylic acid moiety. It is noteworthy that after initial equilibration is complete, the diverse cellular tissues throughout the body show remarkably similar concentrations of activity. Thus, after such equilibration, the largest bulk of [^{11}C]hydantoin activity is located in skeletal muscle, since skeletal muscle is the largest tissue by weight in the body. Such diffuse distribution of hydantoins in the body speak for their relatively uninhibited passage across capillary endothelium and cell membranes, presumably due to their solubility in cell membrane lipoprotein.

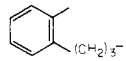
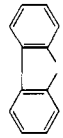
Those hydantoins possessing carboxylic acid moieties such as levulinic acid hydantoin and 2,5-dioxo-4-phenylimidazolidine-4-propionic acid show prominent accumulation in kidneys and excretion in urine as has been previously shown for many other [^{11}C]carboxylates.[1] Such renal concentration and excretion is most probably a function of the organic acid excretory mechanism of renal tubular cells.

While the results described herein were obtained for ^{11}C-labeled hydantoins, similar results would be anticipated with related barbiturates showing similar solubility in, and permeability through, cell membrane lipoprotein.

The use of dilute NaOH as a vehicle for dissolving the compounds described herein is clearly a nonphysiological procedure. Thus, interpretation of the results must be

Table IV. Preparation of Carbon-11 Labeled Spirohydantoins



K^{11}CN, mCi, t_0 (5-mmol rxn scale)	R	Product mCi t_0	Product mCi t_{inj}	Radiochemical yield, %	Chemical yield, %	Total synthesis time, min
607	-$(CH_2)_5$-	344[a]	29	57	55	73
980	-$(CH_2)_6$-	399[b]	26	41	30	81
939	-$(CH_2)_7$-	467[c]	36	50	47	76
1559		932[d]	33	60	81	80
519		253[e]	16	49	70	81

[a] S. D. Upham, U.S. Patent 2 999 863 (Sept 12, 1961) [*Chem. Abstr.*, **57**, 737*e* (1963)], obtained an 87% yield of 5,5-pentamethylenehydantoin on a 1-mol reaction scale by heating cyclohexanone (1 mol), NaCN (1.1 mol), and $(NH_4)_2CO_3$ (4.0 mol) in 25% aqueous ethanol for 4 hr at 94°: mp 215°. In nonradioactive experiments, we obtained a 58% chemical yield of this hydantoin on a 5-mmol reaction scale after heating KCN (5 mmol) and cyclohexanone (5 mmol) and $(NH_4)_2CO_3$ (42 mmol) under pressure at 175–180° for 40 min in 26 ml of 50% aqueous Me_2SO: mp 215–216°. The infrared spectrum of product agreed with Sadtler Prism Spectrum No. 21185. [b] S. D. Upham, see footnote *a*, obtained an 87% yield of 5,5-hexamethylenehydantoin on a 1-mol reaction scale by heating cycloheptanone (1 mol), KCN (1.1 mol), and $(NH_4)_2CO_3$ (4 mol) in 25% aqueous ethanol for 8 hr at 94°: mp 212–213°. In nonradioactive experiments, we obtained a 75% yield of this hydantoin on a 5-mmol reaction scale of KCN and ketone and 42 mmol of $(NH_4)_2CO_3$ upon heating the reagents in 26 ml of 50% aqueous Me_2SO for 40 min at 175–185° under pressure: mp 211–212°. The infrared spectrum of product agreed with Sadtler Prism Spectrum No. 21179. [c] H. C. Brinelow and C. Vasey, British Patent 807 679 (Jan 21, 1959) [*Chem. Abstr.*, **53**, 12304*a* (1959)], prepared 5,5-heptamethylenehydantoin on a 1-mol reaction scale by heating cyclooctanone (1 mol), KCN (2 mol), and $(NH_4)_2CO_3$ (4 mol) in 50% aqueous ethanol for 6 hr at 50–55°: mp 241–242°. In nonradioactive experiments, we obtained this hydantoin in 56% chemical yield upon heating under pressure KCN (5 mmol), cyclooctanone (5 mmol), and $(NH_4)_2CO_3$ (42 mmol) in 26 ml of 50% aqueous Me_2SO for 40 min at 175–185°: mp 242°. The infrared spectrum of product agreed with Sadtler Prism Spectrum No. 26997. [d] L. H. Goodman et al., *J. Org. Chem.*, **25**, 1920 (1960), obtained 1-tetralonespirohydantoin in 52% yield by heating 1-tetralone (0.33 mol), NaCN (0.35 mol), and $(NH_4)_2CO_3$ (100 g) in 60% aqueous ethanol for 8 hr at 56–60° and 1 hr at 85°: mp 241–242.5°. In nonradioactive experiments, we obtained this hydantoin in 53% yield on a 10-mmol reaction scale of KCN and 1-tetralone and 40 mmol of $(NH_4)_2CO_3$ by heating 60 min at 180° under pressure in 25 ml of 50% aqueous ethanol, followed by evaporation to a volume of 10 ml prior to the addition of 40 ml of 2.5% aqueous NaOH, extraction with ether to remove unreacted 1-tetralone, and acidification with concentrated HCl: mp 240–241°. A 45% yield of this hydantoin was obtained after heating KCN (5 mmol), 1-tetralone (5 mmol), and $(NH_4)_2CO_3$ (42 mmol) in 30 ml of 50% aqueous Me_2SO at 190–200° for 40 min: mp 234–235°. The experimental infrared spectrum agreed with Sadtler Standard Prism Spectrum No. 30059. [e] H. R. Henze and R. J. Speer, ADI Document No. 1603, supplement to *J. Am. Chem. Soc.*, **64**, 522 (1942), prepared 9-fluorenonespirohydantoin from fluorenone (20 mmol), KCN (40 mmol), and $(NH_4)_2CO_3$ (80 mmol) by heating in 50% aqueous ethanol at 60° for 2 hr: mp 324–325° dec. In nonradioactive experiments, we prepared this hydantoin in 66% yield on a 5-mmol reaction scale of KCN and 9-fluorenone and 42 mmol of $(NH_4)_2CO_3$ by heating these reagents under pressure in 30 ml of 50–60% aqueous Me_2SO at 190–200° for 40–45 min: mp 324–326° dec.

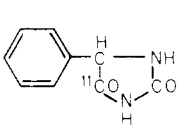
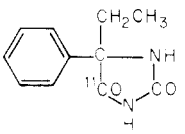
related to the form of administration used as discussed above, and caution must be employed in extrapolating these results to prediction of distribution patterns which one might obtain using conventional routes of administration.

The lack of significant organ concentration (other than that related to initial differences related to physical state and blood perfusion rate) and the lack of rapid excretion of label in urine or bile are consistent with the view that the pharmacologic action of hydantoins largely is related to physical effects on membranes rather than specific chemical interactions with cell constituents. Thus, alterations in their molecular structure which enhance the solubility of the compound in the lipids of biomembranes should enhance their pharmacologic effects. If we use initial accumulation of ^{11}C activity in the brain following intravenous administration of ^{11}C-labeled hydantoins as the model for solubility of the compound in biomembranes, it would appear that such a conclusion is justified, since those hydantoins which in our studies showed the highest initial accumulation in brain generally are those with the greatest reported anticonvulsant effects. This conclusion is of course speculative and is not established by the data presented. Additional work with subcellular components would be necessary to confirm this postulate.

Summary

Five 5,5-dialkylhydantoins, three diarylhydantoins, five 5-aryl-5-phenylhydantoins, and five spirohydantoins were synthesized incorporating the positron emitting, 20.4 min half-life radionuclide, ^{11}C. Total synthesis time was 70–106 min, and 1–59 mCi of final product was obtained. The ^{11}C-labeled hydantoins in 1.0–1.5% aqueous NaOH were administered intravenously and the in vivo distribution pattern was serially imaged using scintigraphic techniques. Variable initial deposition of activity in the vein wall proximal to the site of administration and retention of activity in the lungs were noted, presumably due to precipitation of the compound in the neutral plasma. Hydantoins having two phenyl moieties, and most having one phenyl, and one having a hexamethylene moiety showed initial accumulation of activity in brain. Qualitatively, ^{11}C-labeled dilantin showed the greatest such brain accumulation of activity. Hydantoins having a carboxyl moiety showed prominent renal concentration and urinary excretion. After initial equilibration was complete, except for some moderate concentration of activity in liver, all ^{11}C-labeled hydantoins not possessing

Table V. Relative Carbon-11 Activity in Tissues Following Administration of [^{11}C]Hydantoins[c]

Organ	 [^{11}C]-5-Phenylhydantoin[a]	 [^{11}C]-5-Ethyl-5-phenylhydantoin[b] (nirvanol)
Brain	0.73	1.25
Lungs	1.05	0.75
Heart (washed)	0.71	1.00
Temporal muscle	1.00	1.00
Liver	2.25	1.33
Bile	0.83	1.58
Spleen	0.81	1.25
Stomach	0.76	0.92
Pancreas	0.63	1.67
Kidneys	1.91	1.25
Blood	0.93	1.00
Mesenteric fat	0.25	0.75
Urine		0.67
CSF		2.67

[a] 80 min after intravenous administration. [b] 73 min after intravenous administration. [c] Expressed as cpm/g of tissue/cpm/g of temporal muscle.

a carboxyl moiety showed fairly uniform distribution of label in all cellular tissues of the body. Due to the large skeletal muscle mass in the body, ^{11}C label from hydantoins deposited in skeletal muscle represents the largest extravascular pool of label following completion of initial mixing. No evidence was noted of concentration of label in highly perfused lipid stores such as in omentum or mesentery. The relatively uniform distribution of activity in cellular tissues and slow excretion from the body support the thesis that the pharmacologic action of the hydantoins is related to physical effects on biomembranes rather than to specific chemical interactions with cell constituents.

Acknowledgment. The authors thank Robert Bowman, Michael Garrett, David Heffner, Robert Houston, Yu Chuen Lee, Dennis Lum, Jeffrey Means, Barry Paul, and Eric Schenkel for their contributions to this study. This work was supported by Atomic Energy Commission Contract AT(04-3)-849 to Medi-Physics, Inc., and the Camille and Henry Dreyfus Foundation, Inc., the Du Pont College Science Grant Program, the National Science Foundation Undergraduate Research Participation Program Grant GY-9850, and the National Institutes of Health Grant GM 20073 to Bucknell University. Portions of this paper were presented at the 169th National Meeting of the American Chemical Society before the Division of Medicinal Chemistry, April 8, 1975, Philadelphia, Pa., and at the 19th and 20th Annual Meeting of the Society of Nuclear Medicine, July 12, 1972, Boston, Mass., and June 15, 1973, Miami Beach, Fla., respectively.

References and Notes

(1) M. B. Winstead, J. F. Lamb, and H. S. Winchell, *J. Nucl. Med.*, **14**, 747 (1973).
(2) M. B. Winstead, P. J. Widner, J. L. Means, M. A. Engstrom, G. E. Graham, A. Khentigan, T. H. Lin, J. F. Lamb, and H. S. Winchell, *J. Nucl. Med.*, **16**, 1049 (1975).
(3) J. F. Lamb, R. W. James, and H. S. Winchell, *Int. J. Appl. Radiat. Isot.*, **22**, 475 (1971).

Synthesis of Tetrazole Ribonucleosides and Their Evaluation as Antiviral Agents

Mohindar S. Poonian,* Eugene F. Nowoswiat, John F. Blount, Thomas H. Williams, Ross G. Pitcher, and Michael J. Kramer

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received October 24, 1974*

Synthesis of 1-β-D-ribofuranosyltetrazole and two 5-substituted derivatives, i.e., the 5-carboxamide and 5-acetamide, is described. The stereochemical structure of the parent tetrazole ribonucleoside has been established by means of nuclear Overhauser effect and x-ray crystallography. By analogy to the parent compound, the two 5-substituted tetrazole nucleosides are also assigned the β configuration on the basis of the NMR coupling constant of the anomeric proton and the site of N-ribosylation is determined by ^{13}C NMR studies. Results are also presented on antiviral testing of these synthetic tetrazole nucleosides against influenza A2/Asian/J-305 virus infection in mice.

During the past several years quite a few ribofuranosyl nucleosides in which the base moiety is a five-membered heterocycle have been found to display chemotherapeutic activity. More significant among these are pyrazomycin (I),[1-3] showdomycin (II),[4] and Virazole (Ribavirin) (III)[5] (Chart I). Whereas the first two are *C*-nucleosides, Ribavirin is an example of an *N*-nucleoside. The broad-spectrum antiviral activity of Ribavirin has stimulated interest in the synthesis of ribonucleosides of tetrazole (IV) which is an isosteric ring equivalent of triazole. Although tetrazole derivatives have previously been implicated in certain biological activities[6-8] there is no report hitherto on the synthesis of tetrazole nucleosides or their biological effects.

The present paper describes the synthesis of 1-β-D-ribofuranosyltetrazole and some of its derivatives (IV). The biological screening results against influenza A2/Asian/J-305 virus infection in mice are presented for compounds XIII–XV. Since these are the first examples of tetrazole nucleosides, we have provided rigorous proof of their structural configuration by conducting intramolecular nuclear Overhauser and x-ray crystallographic studies on the parent tetrazole nucleoside (XIII) in addition to proton and ^{13}C NMR studies on all derivatives.

Results and Discussion

Synthesis of Tetrazole Ribonucleosides. The synthesis of tri-O-benzoylated tetrazole nucleosides described here was achieved either by coupling of tetrazole derivatives[9] and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[10] in the presence of mercuric cyanide[11] and nitromethane or, alternatively, by the acid-catalyzed fusion[12] of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and a tetrazole. While the rest of the coupling reactions proceeded to give the expected nucleoside product, in the case of the attempted synthesis of X by the mercuric cyanide method, partial loss of the C-5 carboethoxy substituent was found to have occurred and the resultant coupled product was IX instead (Chart II). Consequently, this approach was abandoned and nucleoside XI was obtained by starting