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Nuclear Medicine Progress Report for Quarter Ending June 30, 1985

F. F. Knapp, Jr.
K. R. Ambrose
M. M. Goodman
P. C. Srivastava

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SUMMARY

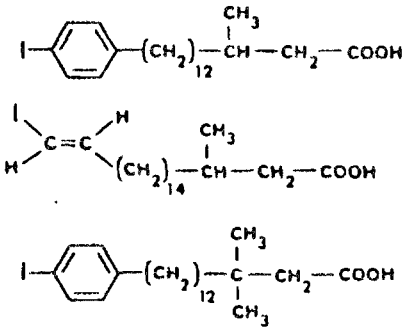
In this report subcellular distribution and lipid analysis studies of the 3-monomethyl-branched (BMIPP), 3,3-dimethyl-branched (DMIPP), and the parent straight-chain 15-para-iodophenylpentadecanoic acid (IPP) are described. Because of the observed differences of the relative myocardial uptake and retention of these agents in fasted and nonfasted rats, a detailed evaluation of the subcellular distribution profiles and the distribution of radioactivity within various lipid pools extracted from cell components was performed. The dimethyl DMIPP analogue showed the longest retention and also showed the highest association with the mitochondrial and microsomal fractions. These data are in contrast to the rapid clearance of the straight chain IPP analogue which showed much lower relative association with the mitochondria and microsomes. The 3-monomethyl (BMIPP) analogue appeared to undergo slower oxidation than the straight chain (IPP), and the dimethyl-branched (DMIPP) analogue was apparently not oxidized by the myocardium. All three analogues showed some incorporation into triglycerides.

Radiolabeled phenylphosphonium cations show high heart uptake and retention and may potentially be a useful class of myocardial perfusion agents. Because the positron emitting radioisotopes of bromine show good radionuclidic properties, a new [^{82}Br]-labeled bromopentenyl-substituted cation has also been prepared and evaluated in rats to determine the effects of bromine substitution within the pentenyl moiety. The model bromovinyl agent was prepared as a mixture of the cis (Z) and trans (E) isomers by Br_2 addition to a vinyl mercuric bromide substrate. The tissue distribution of triphenyl-(E,Z-[^{82}Br]bromopenten-5-yl)phosphonium iodide in rats showed high heart uptake and retention with high heart to blood ratios.

Radioiodinated (E)-2-C-iodovinyl-D-glucose has been prepared as a new iodovinyl-substituted carbohydrate containing C-iodovinyl-branching at the 2-position. For the synthesis of this agent, C-ethynyl-branching was accomplished by preparation of a methyl-α-pyranoside-2-ulose followed by the addition of lithium trimethylsilyl acetylene. The (E)-iodovinyl moiety was fabricated by iododestannylation of a (E)-2-C-(tributyltin)-ethylene carbinol. The ^{125}I-labeled agent was prepared by ^{125}I-I_2 addition to the tin substrate followed by acid hydrolysis.

RADIOIODINATED LONG-CHAIN FATTY ACIDS — EFFECTS OF 3-METHYL-BRANCHING ON INCORPORATION INTO LIPIDS AND DISTRIBUTION IN SUBCELLULAR FRACTIONS FROM RAT HEARTS

To gain insight into the metabolism of the 3-methyl-branched fatty acids, subcellular distribution studies and lipid analyses have been recently performed using the 3-monomethyl (BMIPP) and 3,3-dimethyl (DMIPP)-branched analogues of 15-para-iodophenylpentadecanoic acid (IPP) (Figure 1). Fischer rats were injected intravenously with either



15-(p-Iodophenyl)-3-R,S-Methylpentadecanoic Acid (BMIPP)

E-19-Iodo-3-R,S-Methyl-18-Nonadecenoic Acid

15-(p-Iodophenyl)-3,3-Dimethylpentadecanoic Acid (DMIPP)

Fig. 1. Structures of 3-methyl-branched iodinated fatty acid analogues.

[^{125}I]IPP, [^{125}I]BMIPP or [^{125}I]DMIPP complexed with 6% bovine serum albumin. For subcellular distribution studies, rat hearts were excised in the usual manner and the crude pellet, mitochondrial, microsomal and cytoplasmic fractions obtained by differential centrifugation. Lipids were extracted from either whole heart homogenates or the isolated subcellular fractions, using chloroform:methanol (2:1) by the usual Folch procedure. The extracted lipids were analyzed by thin-layer chromatography using a petroleum ether-ethyl ether-acetic acid (80:20:1) solvent system that adequately separates the various fatty acid pools; polar lipids (R_f = 0.00), diglycerides (R_f = 0.20), free fatty acids (R_f = 0.50), and triglycerides (R_f = 0.75). Sections of the TLC plates were scrapped directly into vials and counted.

Initial studies involved an evaluation of the distribution of radioactivity in the lipid pools from whole hearts of fasted rats up to 1 h after administration of the radioiodinated fatty acid. Injection of the unbranched [^{125}I]IPP analogue resulted in initial high myocardial extraction followed by rapid washout of radioactivity. Lipid analysis demonstrated that the majority of the radioactivity was initially (1-3 min) present in the free fatty acid fraction. There is a rapid increase, however, of radioactivity associated with the triglyceride fraction and this increase is maximal at 10 min (Figure 2). Radioactivity in the diglyceride fraction is maximal at 5 min (20% total activity) and decreased slowly over the remainder of the assay period.

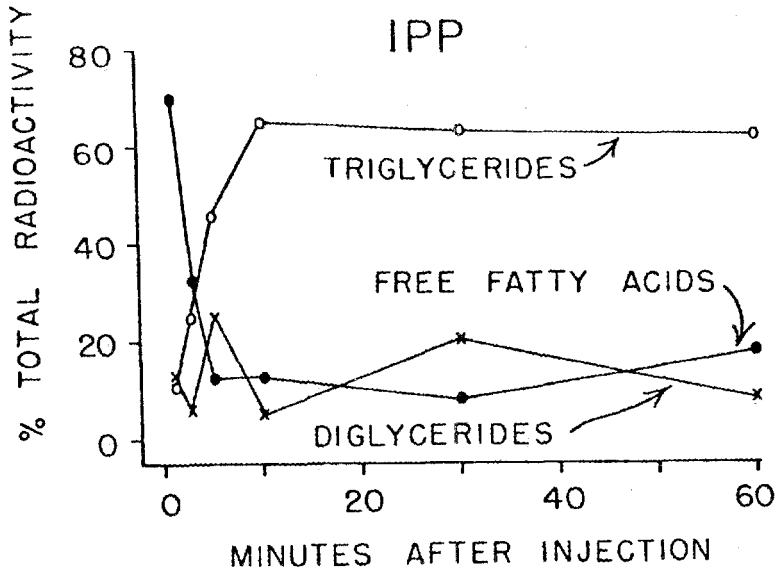


Fig. 2. Distribution of radioactivity in lipid pools isolated by extraction of female Fischer rat hearts following intravenous administration of 15-([^{125}I]iodophenyl)pentadecanoic acid (IPP).

With the BMIPP monomethyl-branched analogue the majority of radioactivity at all assay times was found in the triglyceride fraction with radioactivity in the free fatty acid fraction significant only at the earliest time periods (Figure 3). The radioactivity in the diglyceride fraction was maximal at 3-5 min (14% extracted activity), but fell

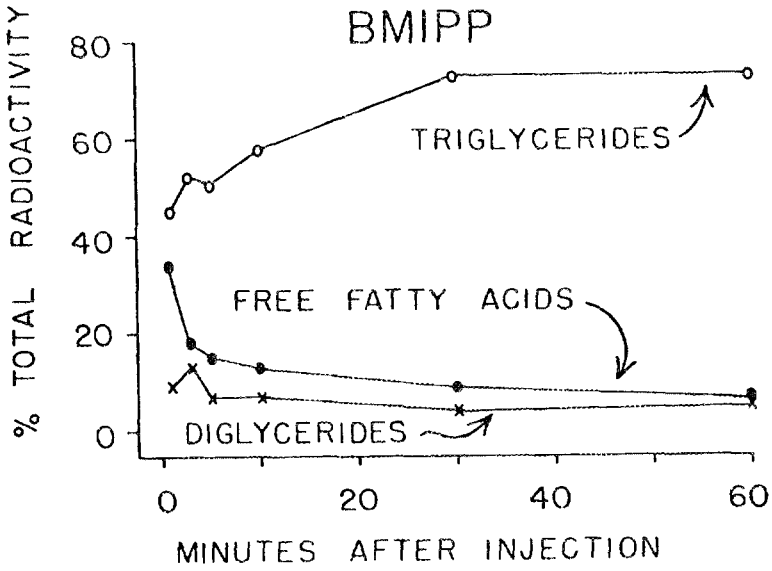


Fig. 3. Distribution of radioactivity in lipid pools isolated by extraction of female Fischer rat hearts following intravenous administration of 15-([^{125}I]iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP).

similar to the activity in the free fatty acid fraction. With the DMIPP dimethyl-branched analogue, the free fatty acid fraction initially contained the majority of radioactivity. At later time periods the triglyceride fraction had the majority of the radioactivity (Figure 4). The rate of "conversion," however, was comparatively slower than with IPP. Similar studies with the three analogues were also conducted with unfasted rats demonstrating a faster incorporation into the triglyceride fraction with IPP and BMIPP and somewhat slower incorporation with DMIPP.

From the preliminary tissue distribution studies, 5 and 30 min time periods were chosen for examination of the lipid pools in the hearts of rats injected with the [^{125}I]IPP/[^{131}I]BMIPP/[^{123}I]DMIPP triple-labeled mixture. This study was designed to eliminate the possible errors inherent in a comparison of the differences that may be detected in subcellular distribution and lipid pools between different groups of rats. In this manner, each measurement has essentially an internal control and differences observed with the three analogues can be

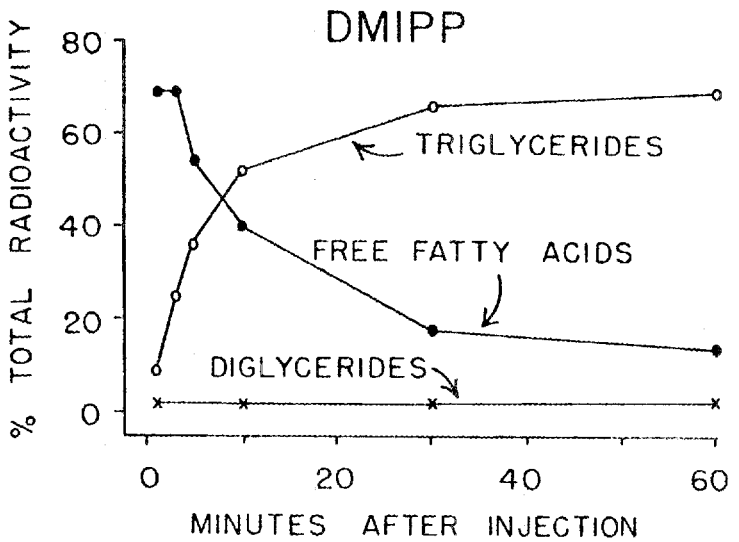


Fig. 4. Distribution of radioactivity in lipid pools isolated by extraction of female Fischer rat hearts following intravenous administration of 15-(p-[^{125}I]iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPP).

directly compared. The mass of each fatty acid injected was very similar to eliminate any effects of possible mass differences on myocardial uptake. The details of this experiment are given in Table 1. For the triple-labeling experiments, the I-123 (159 keV) and I-131 (262 keV) photopeaks were counted simultaneously in two windows and the samples then stored in the cold until the I-123 contribution to the I-125 x-ray photopeak region was less than 4-5%. The samples were then counted again to determine the distribution of I-125. The data from experiments with both fasted and unfasted rats are presented here. The major differences in lipid pools of the three analogues are seen at 5 min post-injection (Fig. 5). At this time the relative proportion of radioactivity in the diglyceride fraction ranks in this order: IPP>BMIPP>DMIPP, whereas the relative distribution of radioactivity in the free fatty acid

Table 1. Summary of experimental details for the evaluation of [^{125}I]IPP/[^{131}I]BMIPP/[^{123}I]DMIPP triple-labeled fatty acid mixture administered to female Fischer rats.

	Radioiodinated fatty acid		
	[^{125}I]IPP	[^{131}I]BMIPP	[^{123}I]DMIPP
Specific activity (μCi/nmole)	2.74	0.47	1.07
μCi/rat	89	15	53
nmole/rat	32.5	32	49.5
μgm/rat	16	16	24

pool is reversed: DMIPP>BMIPP>IPP. At 30 min the majority of radioactivity (64-82%) chromatographed with the triglyceride standard for all three analogues.

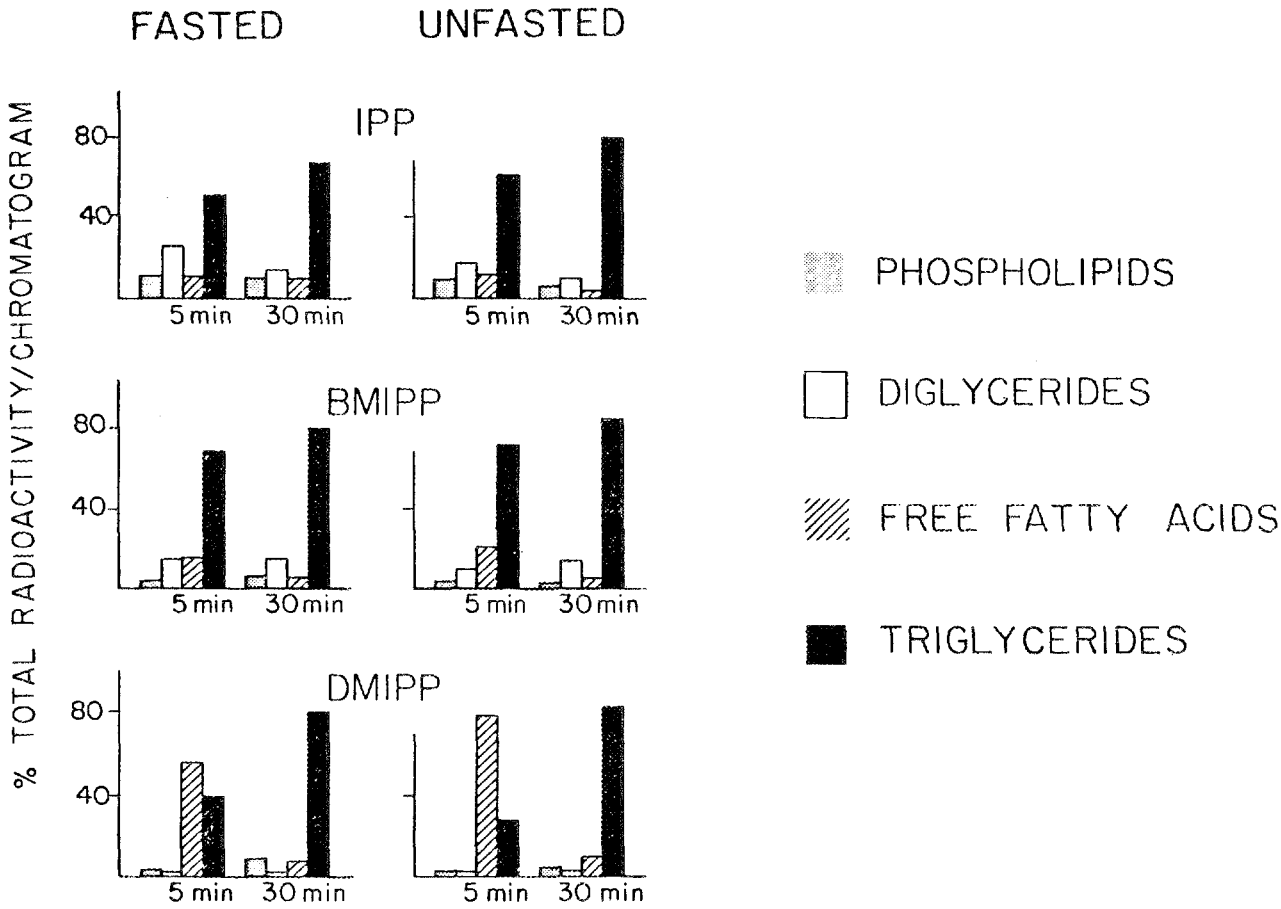


Fig. 5. Comparison of the distribution of ^{125}I, ^{131}I and ^{123}I in lipid pools of heart extracts after administration of the [^{125}I]IPP/[^{131}I]BMIPP/[^{123}I]DMIPP mixture to fasted and unfasted female Fischer rats.

Other investigators have evaluated the myocardial lipid distribution of various radiolabeled fatty acids, including the straight chain IPP agent. In one study, 75-80% of the extractable activity from the hearts of unfasted rats injected with IPP was found in the triglyceride fraction,[1] although no specific assay time was designated. In later reports,[2] between 45-57% of the activity was in the triglyceride fraction with approximately 10% in each of the diglyceride and free fatty acid fractions in fasted rats. These reports compare favorably with the results reported here despite the inability to compare specific assay times. In a study by Otto et al[3] terminally iodinated long chain fatty acids ($I-(CH_2)nCOOH$) where n = 18 or 21 appeared to be fated primarily for triglyceride storage, whereas shorter chain fatty acids ($n \leq 15$) were subject to β-oxidation. Since the fatty acids in this study were without the phenyl moiety and were not branched, it is difficult to draw comparisons; however, it appears that the phenylpentadecanoic analogues have a chain length that would favor triglyceride storage.

To further investigate the metabolism of the 3-methyl-branched and straight chain analogues, subcellular distribution experiments were performed in independent studies with extracts of hearts excised from fasted and nonfasted rats 30 min after injection of the [^{125}I]labeled analogues. Comparison of the results obtained in nonfasted rats (Figure 6) shows little difference in the relative subcellular distribution of each compound at 30 min. In rats fasted for 24 h, however, there are major differences in the subcellular distribution profiles of the branched and unbranched analogues in the subcellular fractions isolated 30 min after injection. The IPP straight chain analogue, which shows rapid washout in the hearts of fasted rats, resulted in a proportionally greater amount of radioactivity in the cytoplasmic fraction with the corresponding proportional lower activity in the mitochondrial and microsomal fractions. Although this subcellular distribution profile of IPP obtained in fasted rats was significantly different from that observed with unfasted rats, comparison of the % dose/fraction values demonstrated similar levels of IPP in the cytoplasm. Thus, the apparent increase of radioactivity in the cytoplasm of the hearts of fasted rats

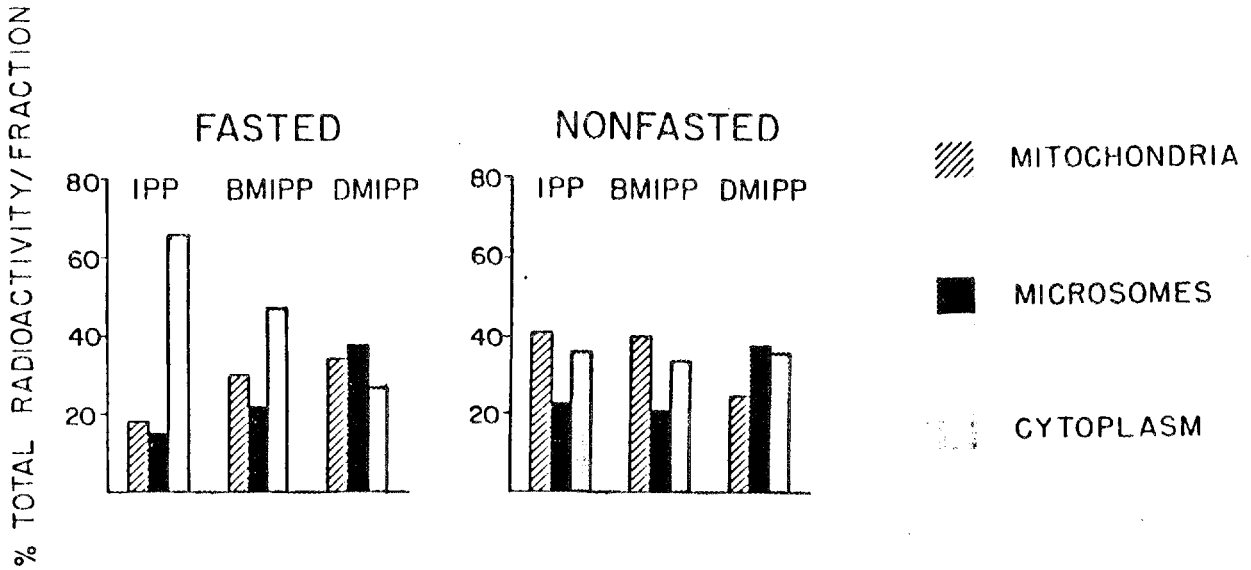


Fig. 6. Distribution of radioactivity in subcellular fractions from heart homogenates 30 min after intravenous administration of either [^{125}I]IPP, [^{125}I]BMIPP or [^{123}I]DMIPP to separate groups of fasted and nonfasted female Fischer rats.

is actually a proportional increase due to the loss of activity from the mitochondrial and microsomal fractions. The monomethyl-branched BMIPP analogue, which shows longer myocardial retention in vivo than IPP, showed comparatively higher percentages of radioactivity in the mitochondrial and microsomal fractions. The DMIPP dimethyl-branched analogue, which shows the longest in vivo myocardial retention of the three analogues, also had the highest proportion of radioactivity associated with the microsomal and mitochondrial fractions. From these results and from previous studies in which the subcellular distribution profiles of a number of fatty acids were compared, it appears that the length of retention in the hearts of fasted rats correlates with the relative proportion of radioactivity found in the mitochondrial and microsomal fractions 30 minutes after injection.

These subcellular studies were expanded to include both the 5 and 30 min assay periods and also lipid analysis of the subcellular fractions. Because the 3-4 day period required to complete these analyses made it impractical to use ^{123}I-labeled fatty acids, ^{131}I/^{125}I-labeled mixtures

of two analogues [^{131}I]IPP/[^{125}I]DMIPP and [^{131}I]BMIPP/[^{125}I]DMIPP) were evaluated in fasted rats in two separate experiments. The comparative differences in the 30 min subcellular profiles observed in the earlier experiments were reproduced in these dual label experiments, and the subcellular distribution patterns at 5 min resembled those found at 30 min. It thus appears there are no shifts in the relative proportion of radioactivity within the subcellular fractions between 5 and 30 min (Figure 7). As might be expected from previously described lipid analysis of "whole hearts," however, there were differences observed in

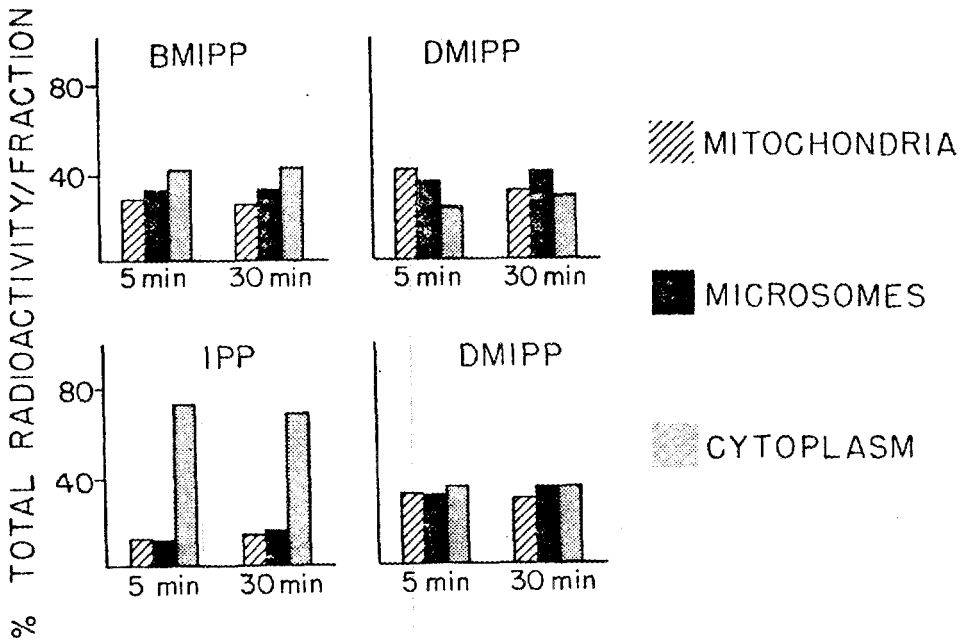


Fig. 7. Comparison of the subcellular distribution of ^{125}I and ^{131}I from heart homogenates at 5 min and 30 min following the intravenous administration of either [^{131}I]IPP/[^{125}I]DMIPP or [^{131}I]BMIPP/[^{125}I]DMIPP mixtures to fasted female Fischer rats.

the lipid pools within these cellular fractions at the different time intervals. Lipid analysis of the pellet of the centrifugation of the crude homogenate (see Figures 8 and 9) was performed and may approximate the

lipid profile of unfractionated heart tissue. With all cell fractions for the three analogues, there was a shift to a predominance of radioactivity in the triglyceride fraction 30 min after injection (Figures 8 and 9). The comparison of radioactive lipid pools between the different cell fractions of hearts of rats injected with the same compound or the comparison of the same subcellular component of hearts with different compounds injected, however, showed a number of observable differences.

With the straight-chain IPP analogue (Figure 8), the radioactive lipid profiles of the microsomal and crude pellet fractions are quite

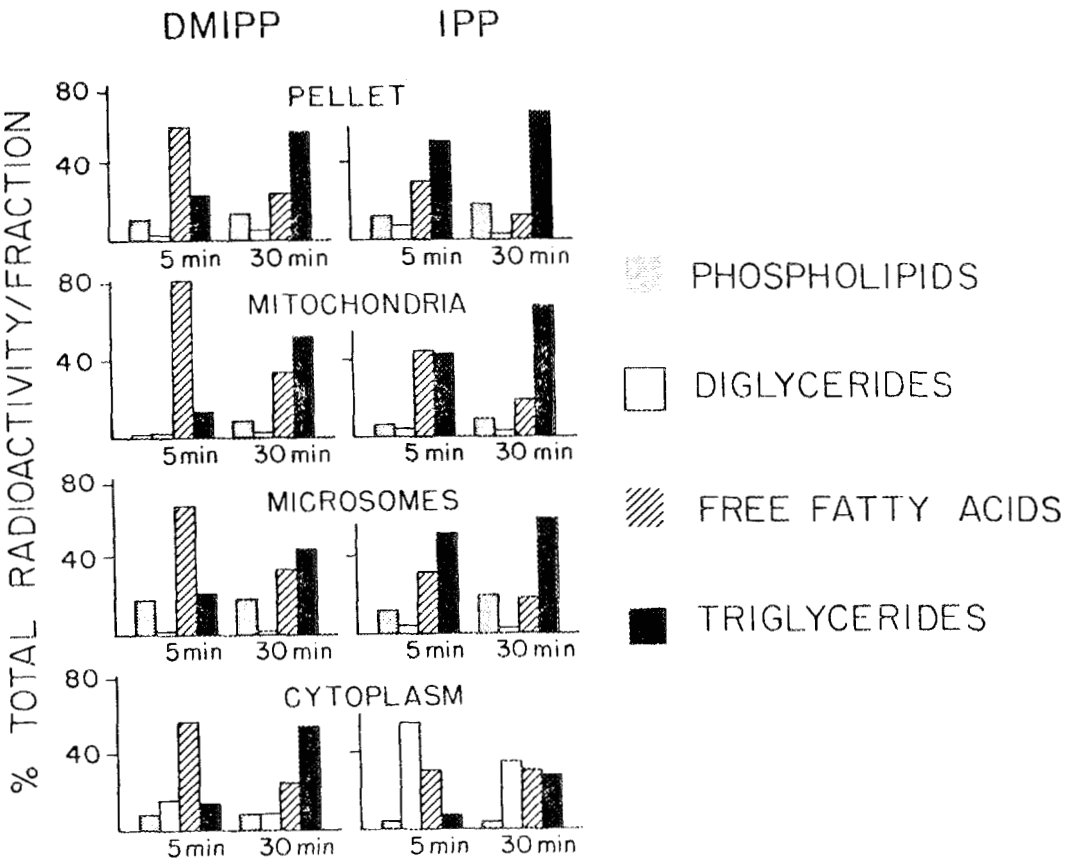


Fig. 8. Distribution of radioactive lipids from the subcellular fractions of the [^{131}I]IPP/[^{125}I]DMIPP study described in Figure 7.

similar, whereas the mitochondrial fraction shows a greater proportion of free fatty acid. In the cytoplasmic fraction 5 min after injection, the majority of the extractable radioactivity chromatographs with the diglyceride standard. With the monomethyl-branched BMIPP analogue (Figure 9), the distribution of radioactive lipids in the microsomal fraction again resembles the crude pellet. For both the cytoplasmic and mitochondrial fractions, however, radioactivity in the free fatty acid component becomes a more predominant feature of the lipid profile particularly 5 min after injection. With DMIPP (Figures 8 and 9) both the microsomal and cytoplasmic fractions of rat hearts injected with this dimethyl-branched fatty acid show lipid profiles similar to the crude pellet. In the mitochondrial fractions from these hearts, however, almost 80% of the extractable activity is in the form of free fatty acid.

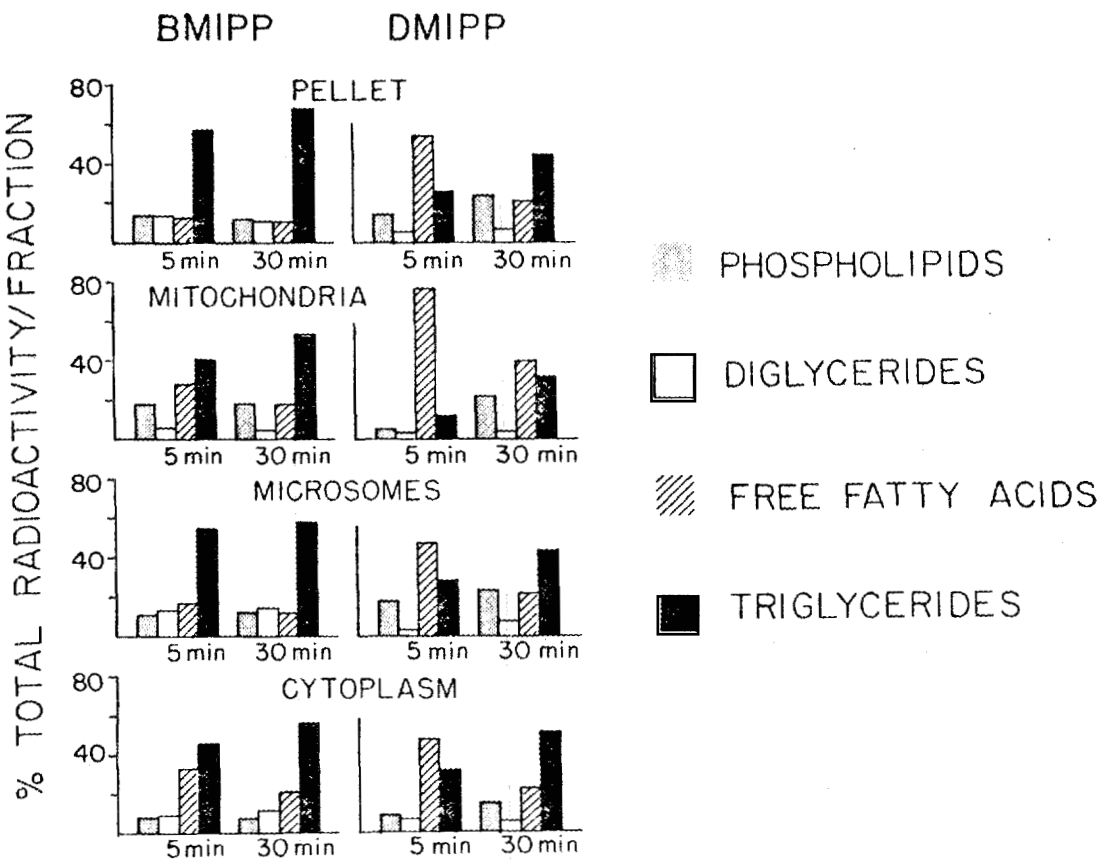
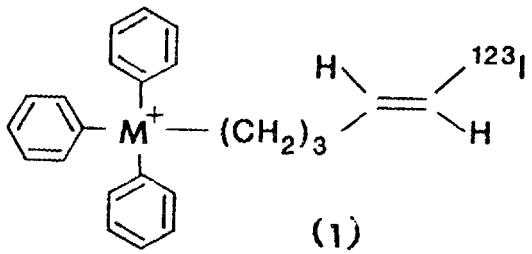


Fig. 9. Distribution of radioactive lipids from the subcellular fractions of the [^{131}I]BMIPP/[^{125}I]DMIPP study described in Figure 6.

An evaluation of the subcellular distribution profiles and the distribution of radioactive lipids extracted from the cell components shows distinct differences in the three analogues. Both the relative association within the mitochondrial versus the cytoplasmic fractions and the lipid profile of these two cell components appear to be the major differences that can be correlated between the structures of these analogues and the observed differences in their in vivo behavior. The IPP straight chain analogue shows poor myocardial in vivo retention and its metabolic products seem to be primarily associated with the cytoplasm, initially found predominantly in the diglyceride pool. At the other extreme, DMIPP shows the best myocardial retention of the three analogues and shows an early association with the mitochondria where the extractable radioactivity is almost exclusively in the free fatty acid fraction. Finally, BMIPP shows primarily equal distribution in the mitochondrial, microsomal and cytoplasmic fractions and seems to demonstrate the most rapid incorporation into triglycerides. Further analyses and identification of metabolites is being pursued. The present studies demonstrate clear differences in the metabolic basis for myocardial retention of these methyl-branched fatty acids.

RADIOLABELED MYOCARDIAL IMAGING AGENTS — RADIOBROMINATED PHOSPHONIUM CATIONS

The design and synthesis of various phosphonium cations have been described in earlier reports in this series (ORNL/TM-8619, -8827, -9037, -9343). These agents show high specificity and good retention due to the unique cationic properties. Several cationic compounds of phosphorus, arsenic and nitrogen were prepared with radioiodide stabilized as a terminal vinyliodide (1). All these analogues showed high heart uptake and good heart:blood ratios. Excellent myocardial images were obtained with (1, M=P) in dogs, but this agent exhibited relatively rapid myocardial washout. The radiobrominated vinylalkyl phosphonium cation analogue (5) has now been prepared using synthetic techniques described earlier (ORNL/TM-8746) for evaluation in rats and for structure determination by single crystal X-ray analysis. The

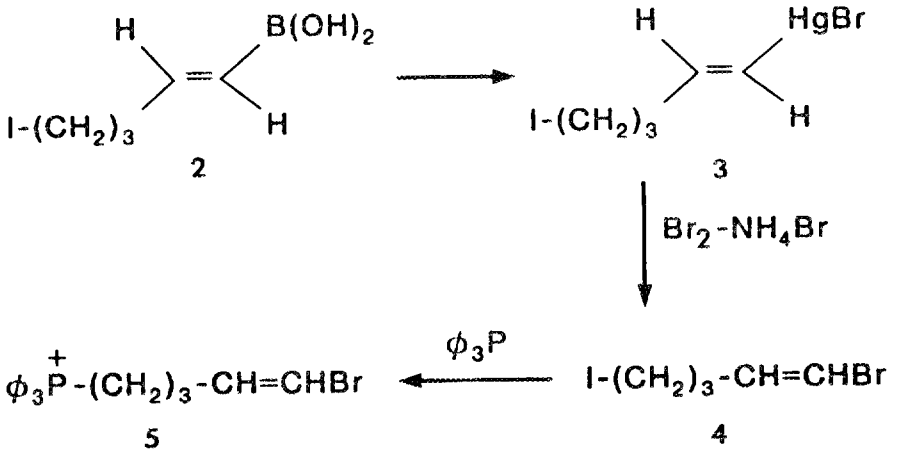


(1)

synthesis of (5) is described in Scheme I. The preparation of this bromine analogue has been pursued because of the potential use of bromine radioisotopes. Bromine-75 is a positron emitter with a half-life of 101 min and decays with a high positron yield (76%), and bromine-76 ($T_{1/2}$ = 15.9 h) is also useful for positron emission tomography (PET) and decays with a high positron yield (57%). Both of these radionuclides can be produced in medical cyclotrons and there is widespread interest in their use in positron emission tomography. For preliminary developmental studies the readily available ^{82}Br radioisotope ($T_{1/2}$ = 13 h) was used.

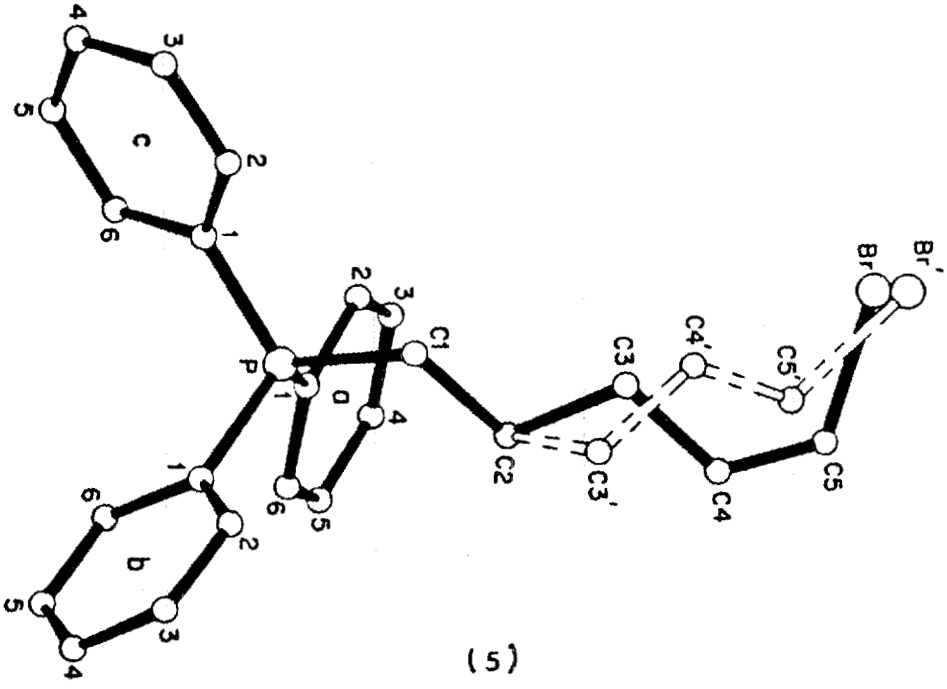
For the preparations of the model bromovinyl phosphonium cation, the iodovinylboronic acid (2) was converted into the corresponding vinylmercury bromide (3). The vinylmercury bromide intermediate was then treated with molecular bromine (Br_2) in benzene to yield the corresponding vinylbromide (4). By equilibrating radiobromide ($^{82}Br^-$) with Br_2 prior to addition to the vinylmercuric bromide substrate (3), the radiobrominated product (4) was obtained. In developmental studies the unlabeled vinylbromide product (4) obtained by this technique was analyzed by ^1H nuclear magnetic resonance spectroscopy and found to be a mixture of the cis (Z) and trans (E) isomers.

Further conclusive proof for the structure of (4) was estabished by the single crystal X-ray analysis of (5) prepared by the condensation of (4) with triphenylphosphine in benzene. The single crystal X-ray analysis of (5) was perfomed by Barry M. Goldstein, M.D., Ph.D., a collaborator at the University of Rochester Medical Center. The molecular structure is illustrated in Fig. 10. The crystal is composed



Scheme I

of a mixutre of 2/3 cis and 1/3 trans conformers with random site occupancy. The alkyl chain is extended in both cases, the Br and Br^1 positions lying within 0.5 A of each other. The P-C1-C2 bond angle is 120.9(5)°, a significant deviation from the tetrahedral angle of 109.5°. This is probably due in part to a distortion in the P...C2 distance introduced by the restraint placed on the C1-C2 bond. However, large distortions from tetrahedral angles are not uncommon about sp^3 hybridized alkyl chain carbons. The P-C1 bond length of 1.773(7)A falls within the rather wide range of observed Ph_3P-C(sp^3) bond lengths (e.g., 1.755(3)A. The P-C(sp^2) bond lengths are within the expected range and the bond angles about the phosphorus atom show only small deviations from a tetrahedral geometry. The phenyl groups are planar within experimental error. The triphenylphosphonium moiety shows significant deviations from C_3 symmetry, ring A being rotated about the P-C(sp^2) bond to a greater extent than rings B and C. On the basis of a survey of a large number of triphenylphosphine structures, Bye, Schweizer and Dunitz[4] have proposed a pathway for the stereoisomerization of an equilibrium C_3-symmetric structure to its enantiomer. The triphenylphosphonium geometry observed here (o_a = -65.9(5)°, o_b = +18.1(4)°, o_c = -22.7(6)°) would lie along this pathway roughly between



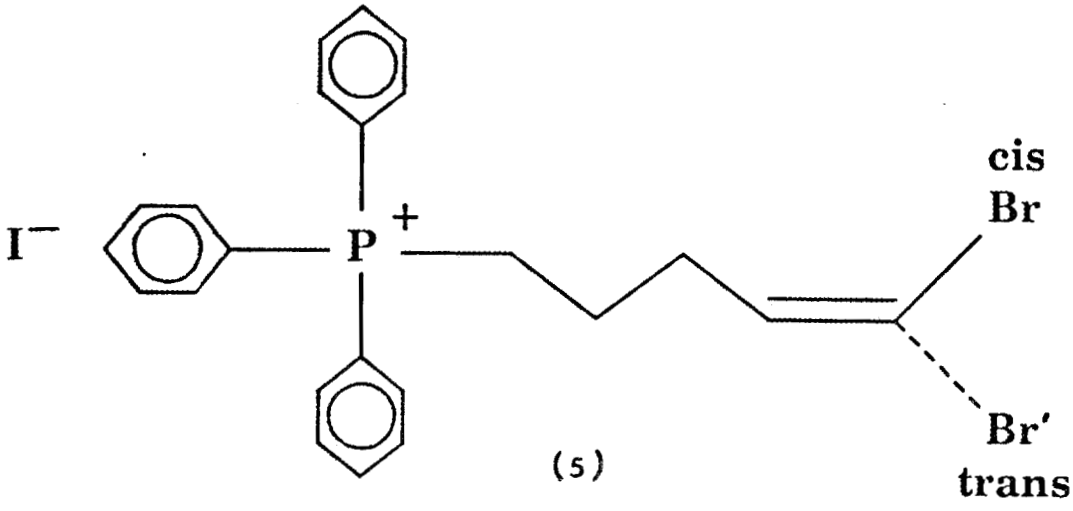


Fig. 10. Single crystal X-ray structures of the cis-trans vinyl bromide (4) mixture (courtesy of Barry M. Goldstein, M.D., Ph.D., University of Rochester Medical Center).

the C_3-symmetric structure and a putative transition state structure with torison angles o_a = -90°, o_b = +10°, o_c = -10°. The iodide anion sits in a pocket bounded on two sides by alkyl chains of neighboring molecules.

The tissue distribution of the cis-trans mixture of radiobrominated (5) was determined in rats (Table 2). The data indicate high myocardial extraction and high heart:blood ratios. Compound (5) showed a gradual increase in myocardial retention over a 120 min period (% dose/gm: 5 min, 4.5; 120 min, 5.75) as compared to (1, M=P) in which case a gradual decrease in myocardial retention (% dose/gm: 5 min, 5.5; 120 min, 3.87; ORNL/TM-8619) was observed. If these data can be directly correlated with myocardial retention in dogs, the radiobrominated agent (5) might be a superior candidate than (1) for further evaluation as a myocardial imaging agent.

Table 2. Distribution of radioactivity in tissues of Fischer 344 female rats following the administration of triphenyl-(E,Z-[^{82}Br]bromopenten-5-yl)phosphonium iodide mixture (5).

Time after injection (min)	Mean percent injected dose/gm (range)						
	Heart	Blood	Liver	Lung	Kidney	Thyroid	Heart: Blood (Mean)
5	4.50 (3.53-5.82)	0.21 (0.14-0.27)	0.94 (0.57-1.36)	1.58 (1.29-1.92)	8.50 (0.39-12.86)	28.00 (23.35-32.92)	21.69
30	5.26 (4.69-5.59)	0.14 (0.13-0.15)	0.30 (0.25-0.34)	1.58 (1.41-1.75)	8.66 (7.07-10.16)	28.82 (24.73-34.29)	37.62
60	5.48 (4.28-7.55)	0.14 (0.12-0.16)	0.17 (0.15-0.18)	1.33 (1.12-1.59)	5.76 (4.96-6.79)	23.25 (19.26-31.96)	39.40
120	5.75 (5.07-7.35)	0.14 (0.12-0.17)	0.12 (0.09-0.13)	1.16 (0.96-0.13)	3.68 (2.50-5.77)	21.19 (18.21-24.71)	40.40

RADIOIODINATED BRANCHED CARBOHYDRATES — POTENTIAL NEW AGENTS TO EVALUATE CEREBRAL AND MYOCARDIAL DISORDERS

In a recent report (ORNL/TM-9480) the synthesis and tissue distribution in rats of a model radioiodinated carbohydrate containing radioiodide stabilized as an iodovinyl moiety (E)-C-3-[^{125}I]iodovinyl-D-allose (Fig. 11) were described. This study was performed to develop

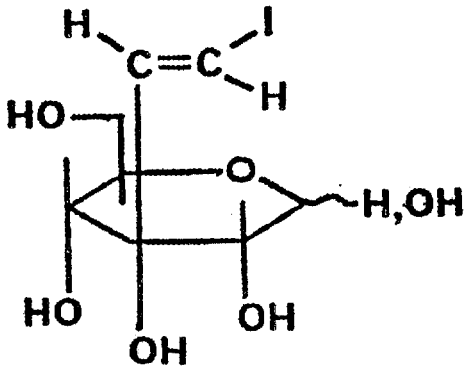
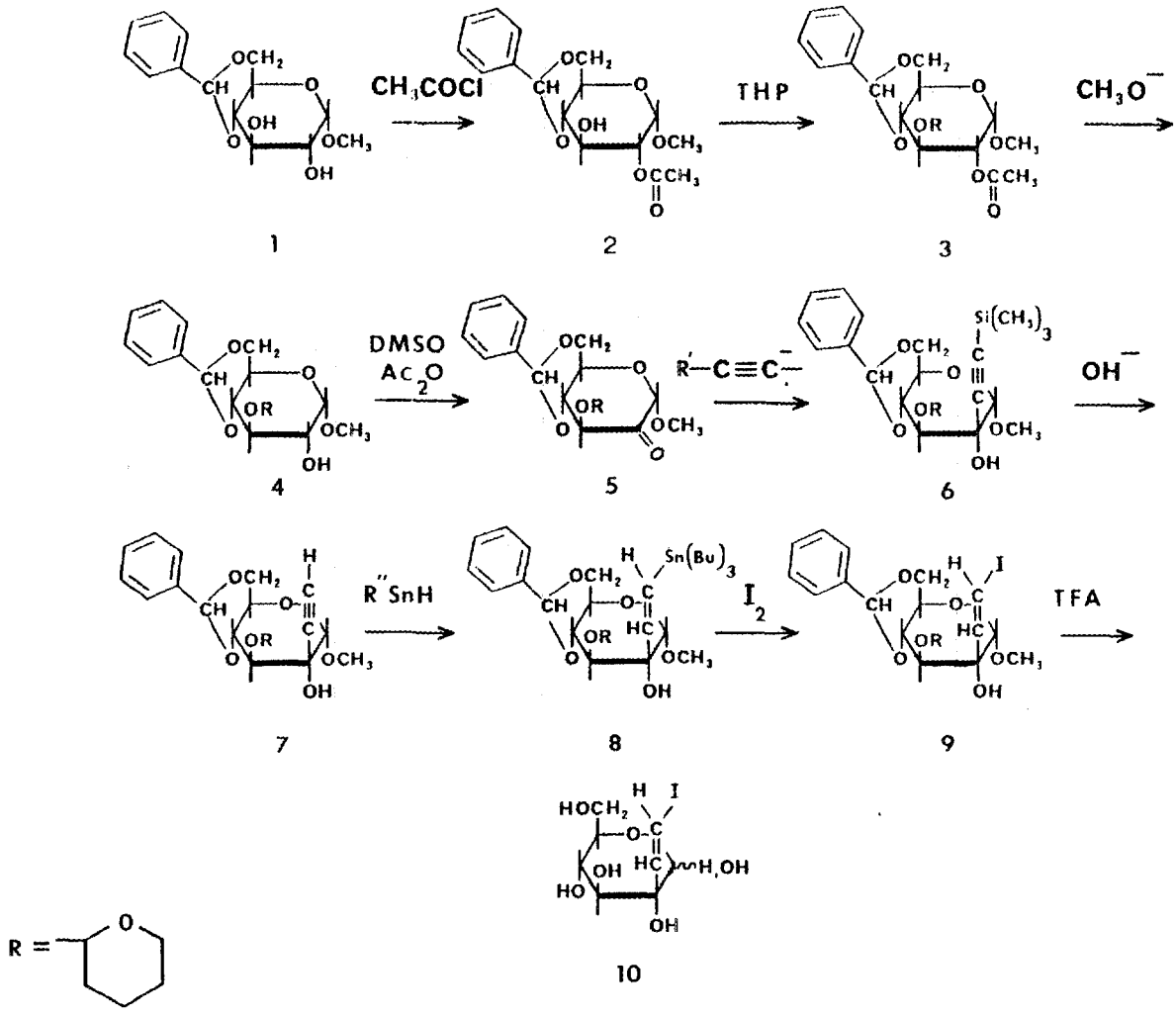


Fig. 11. Structure of (E)-C-3-iodovinyl-D-allose.

an iodine-123-labeled glucose analogue designed to metabolically trap the radioiodide and enable the regional study of glucose metabolism in the brain and heart by single photon tomographic (SPECT) techniques. Tissue distribution in rats with (E)-C-3-iodovinyl-D-allose showed low heart and brain uptake. On the other hand, the radioiodinated agent did show low in vivo deiodination which resulted in low thyroid uptake (60 min, 6.07% dose/g). Thus, it was demonstrated for the first time that radioiodide can be stabilzed on a carbohydrate by attachment to the sp^2-hybridized carbon of a vinyl moiety. Because the model radioiodinated agent did not have the active D-gluco configuration, the low brain uptake may be attributed to low affinity for the carrier system required for sugar transport across the blood-brain barrier. With these results at hand, the synthesis of a C-iodovinyl carbohydrate whereby the hydroxyl groups have the all trans or gluco configuration in (E)-C-2-iodovinyl-D-glucose is now reported.

The synthetic approach for the preparation of the gluco C-iodovinyl-branched carbohydrate involved the attachment of an ethynyl function onto the 2-position of the pyranose ring via the addition of lithium trimethylsilyl acetylene to a keto intermediate. Methyl 4,6-O-benzylidene-3-O-methyl-α-arabino-hexopyranoside-2-ulose (5)[5] was selected (Scheme II) as the keto intermediate because the anomeric configuration of the substate will result in the approach of the negatively charged acetylide to the C-2 carbonyl carbon in such a manner to afford the desired gluco ethynyl carbinol (6). In addition, the blocking groups of 4,6-O-benzylidene-3-O-methyl-α-arabinohexopyranoside-2-ulose (5) are stable under the basic conditions used to introduce the ethynyl substituent at C-2 but are later readily removed by dilute acid. In this synthetic approach the key intermediate 5 was prepared in a four-step sequence of reactions outlined in Scheme II. Commerically available methyl 4,6-O-benzylidene-α-D-glucopyranoside (1) was treated with acetyl chloride in pyridine to afford methyl 2-O-acetyl-4,6-O-benzylidene-α-D-glucopyranoside (2). Treatment of the 2-acetate with 3,4-dihydro-2H-pyran gave methyl 2-O-acetyl-3-O-(tetrahydropyran-2-yl)-α-D-glucopyranoside (3). The acetyl group of the tetrahydropyranyl ether 3 was removed by treatment with methoxide. The 3-hydroxyl function of tetrahydropyranyl ether (4) was then oxidized under Albright-Goldman conditions using methyl sulfoxide-acetic anhydride to give the desired 2-ulose (5). The pivotal step in the synthesis of 2-C-iodovinyl glucose (10) involved introduction of the acetylene function via addition of the anion of trimethylsilyl acetylene. Methyl 2-C-trimethylsilylethynyl-3-O-(tetrahydropyran-2-yl)-4,6-O-benzylidene-α-D-glucopyranoside (6) was treated with methanolic-NaOH to afford the desired 2-C-ethynyl derivative (7).

Iodide was introduced into the geminal position of the ethynyl moiety by iododestannylation of the tributyl tin intermediate (8). Hydrostannylation of the ethynyl substrate (7) with tributyltin hydride gave the (E)-2-C-(tributyltin)ethylene derivative (8). Treatment of the tin substrate (8) with iodine in methylene chloride followed by dilute acid gave (E)-2-C-iodovinyl-D-glucose. The proton magnetic resonance (PMR)



Scheme II

spectra of (9) and (10) exhibited a characteristic AB doublet centered at 6.67 (J = 2 Hz) corresponding to the olefinic protons. A comparison of the chemical shifts of the anomeric protons in the alkynyl intermediates (6) and (7) with that in the olefinic compounds (8) and (9) enables assignment of the gluco-configuration to compounds (6)-(9). The trimethylsilylacetylene intermediate (6) and the ethynyl intermediate (7) have two proton signals each corresponding to a single proton at δ 5.5, δ 5.6 and δ 5.53, δ 5.6, respectively. The proton attached to the benzylidene function is expected to be the most deshielded and is readily identified as having a chemical shift of δ 5.6. The remaining two peaks at δ 5.5 and δ 5.53 can be assigned in the following manner. The anomeric proton of a methyl α-glucopyranoside bearing an alkynyl function in the 2-position is expected to resonate at a more deshielded position than the normal frequency of δ 4.75-4.85. The anomeric proton of the glucopyranoside will be situated perpendicular to the secondary field caused by the anisotropy of the acetylenic linkage and should experience a deshielding effect. Thus, the signals of δ 5.53 and δ 5.5 in the PMR spectra (6) and (7) can be assigned to H-1 of a methyl α-glucopyranoside. The assignment is further confirmed by a similar examination of the chemical shifts from the benzylidene and anomeric protons of the olefinic compounds (8) and (9). The benzylidene proton of (8) and (9) absorb at δ 5.6 while the anomeric proton no longer shows a signal in the δ 5-6 region. The PMR spectra of (8) and (9) do show new resonances at a more shielded position δ 4.63. The elimination of the deshielding influence of the alkynyl linkages by derivatization to olefinic moieties as shown in the spectal data supports the α-glucopyranoside configurational assignment.

The radioiodinated E-C-2-iodovinyl-D-glucose was prepared by $^{125}I_2$ treatment of (8) as described for the unlabeled compound. Tissue distribution studies in female Fischer rats will be performed and the results reported in a future technical report.

AGENTS FOR MEDICAL COOPERATIVE PROGRAMS

Osmium-191

Nine shipments of ^{191}Os-potassium osmate were made for the Medical Cooperative Programs for further development of the ^{191}Os→^{191m}Ir generator system and to study clinical applications of ^{191m}Ir ($T_{1/2}$ = 4.96 s). Applications being studied include first-pass radionuclide angiographic evaluation of intracardiac shunts, measurement of left ventricular ejection fraction and evaluation of regional myocardial perfusion under constant infusion conditions. Two shipments were made to the Children's Hospital Medical Center in Boston, MA (Drs. S. Treves and A. Packard), four shipments to the Cyclotron Center, University of Liege, Belgium (Drs. C. Brihaye and M. Guillaume), one shipment to Harbor UCLA Medical Center in Torrance, CA (Dr. E. Mena), one shipment to George Washington University, Washington, D.C. (Dr. B. Wessels), and one shipment to Massachusetts General Hospital, Boston, MA (Dr. H. W. Strauss).

Platinum-195m

One shipment of ^{195m}Pt-labeled cis-dichlorodiammineplatinum(II) (cis-DDP) was supplied to the University of Southern California (Dr. W. Wolf) to study the pharmacokinetic properties and mode of antitumor activity of this agent.

Copper-64

Two shipments of ^{64}Cu-citrate were made to collaborators at the Oak Ridge Associated Universities (Dr. J. Crook).

LIST OF REFERENCES

1. S. N. Reske, H.-J. Machulla and C. Winkler, J. Nucl. Med., 23, P 10 (1982).
2. S. N. Reske, W. Saver, H.-J. Machulla and C. Winkler, J. Nucl. Med., 25, 1335-1342 (1984).
3. C. A. Otto, L. E. Brown, D. M. Wieland and W. H. Beierwaltes, J. Nucl. Med., 22, 613-618 (1981).
4. E. Bye, W. B. Schweizer and J. D. Dunitz, J. Amer. Chem. Soc., 104, 5893 (1982).
5. A. N. DeBelder and E. Wiren, Carbohyd. Res., 24, 166-168 (1972).

PAPERS AND PUBLICATIONS

Publications

M. M. Goodman, A. P. Callahan, and F. F. Knapp, Jr. "Design, Synthesis, and Evaluation of ω-Iodovinyl- and ω-Iodoalkyl-Substituted Methyl-Branched Long-Chain Fatty Acid," J. Med. Chem., 28, 807-815, 1985.

P. C. Srivastava, F. F. Knapp, Jr., A. P. Callahan, B. A. Owen, G. W. Kabalka, and K. A. R. Sastry "Myocardial Imaging Agents: Synthesis, Characterization and Evaluation of (Z)- and (Z,E)-18-[^{82}Br] Bromo-5-tellura-17-octadecenoic Acids," J. Med. Chem., 28, 408-413, 1985.

P. C. Srivastava, F. F. Knapp, Jr., G. W. Kabalka and S. A. Kunda "Facile - Radioiodemetallation Reactions for the Convenient Preparation of Radioiodinated Compounds," Synth. Comm., 15, 355-364 (1985).

Reports

F. F. Knapp, Jr., K. R. Ambrose, M. M. Goodman, and P. C. Srivastava, Nuclear Medicine Progress Report for Quarter Ending March 31, 1985, ORNL/TM-9609.

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