Relationship of Molecular Structure to in Vivo Scintigraphic Distribution of Carbon-11-Labeled Compounds. 4. Carbon-11-Labeled Mandelonitriles, Mandelic Acids, and Their Esters

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^{11}C-Labeled HCN was collected in water containing carrier KCN following bombardment of 99% N_2–1% H_2 with 22 MeV protons. ^{11}C-Labeled mandelonitrile and *p*-methoxy-, *p*-hydroxy-, and 3,4-dihydroxymandelonitrile were synthesized from K^{11}CN and the corresponding benzaldehyde. The initial distribution of ^{11}C activity of these nitriles in dogs was primarily in the region of the heart, liver, and kidneys followed by rapid redistribution to the parotids and stomach with the [^{11}C]hydroxymandelonitriles. ^{11}C-Labeled mandelic acid and *m*-methyl-, *o*-chloro-, and *p*-chloromandelic acid were synthesized from the corresponding [^{11}C]mandelonitrile. Serial scintigraphy of ^{11}C activity of these mandelic acids in dogs showed progressive renal excretion with accumulation of activity in the bladder. ^{11}C-Labeled ethyl and benzyl mandelate were synthesized from [^{11}C]mandelic acid. These esters showed initial accumulation of activity in the lungs with eventual excretion by the kidneys.

Mandelonitrile has been proposed as the "active antitumor" principal arising from the hydrolysis of amygdalin,[1] and mandelonitrile derivatives have been employed as precursors in the synthesis of ^{11}C-labeled neurohumeral transmitters.[2] Mandelic acid has been used clinically as a urinary antiseptic,[3] and esters of mandelic acid have been shown to possess antispasmodic properties.[4]

The diverse pharmacologic properties of this family of related compounds and the ability to achieve their rapid synthesis incorporating positron-emitting, 20.4-min half-life ^{11}C attracted our attention. The results of their synthesis and in vivo evaluation are the subject of this communication. In previous articles we reviewed the rationale for employing serial scintigraphy for studying the distribution of families of related ^{11}C-labeled compounds.[5-7]

Experimental Section

Materials and Methods. ^{11}C-Labeled mandelonitrile[8a] and *p*-methoxy-,[8b] *p*-hydroxy-,[9] and 3,4-dihydroxymandelonitrile[10] were prepared from K^{11}CN and the corresponding benzaldehyde–bisulfite addition product and were dissolved in ethanol or water prior to intravenous administration to the dog. The ^{11}C-labeled mandelic acids were synthesized by modifying the procedure for preparing [^{14}C]mandelic acid.[11] H^{11}CN was produced on bombardment of 99% N_2–1% H_2 with 30–40 μA of 22 MeV protons for 60–90 min.[12] A solution of K^{11}CN formed by exchange between H^{11}CN and 5 mmol of carrier KCN in water was added to 7.5 mmol of the appropriate benzaldehyde. The reaction mixture was kept below 5 °C while 1.1 g of $NaHSO_3$ in water was added followed by stirring for 20 min. The mandelonitrile derivative was extracted with benzene and added to 20 mL of concentrated hydrochloric acid. The mixture was boiled to dryness, the residue extracted with hot benzene, and the extract filtered and added to hexane. Upon cooling the [^{11}C]mandelic acid crystallized and was dissolved in 7.5% $NaHCO_3$ prior to administration to the dog.

^{11}C-Labeled ethyl mandelate was prepared as follows. ^{11}C-Labeled 2,2-dimethyl-5-phenyl-1,3-dioxolan-4-one was prepared from [^{11}C]mandelic acid and acetone.[13] This product was dissolved in ethanol, hydrogen chloride gas was briefly bubbled into the solution and evaporated, and the residue of [^{11}C]ethyl mandelate was dissolved in Me_2SO prior to its injection to the dog.

^{11}C-Labeled benzyl mandelate was prepared as follows. A solution of 1-*p*-tolyl-3-benzyltriazine in benzene was added to a solution of ^{11}C-labeled mandelic acid in benzene.[14] The reaction mixture was heated to boiling, then cooled, and extracted successively with 10% HCl and 10% Na_2CO_3. Following evaporation of the benzene, the residue of [^{11}C]benzyl mandelate was recrystallized from hexane, dissolved in Me_2SO, and administered to the dog.

Prior to the ^{11}C reaction each mandelonitrile, mandelic acid, and ester was prepared from nonradioactive cyanide under experimental conditions designed to optimize the yield at minimal reaction times. The resulting products were isolated and purified, and their melting points and infrared and NMR spectra were determined as a confirmation of their respective data reported in the literature.

The animals were fasted overnight before being surgically anesthetized with nembutal. The in vivo distribution of the intravenously administered ^{11}C compounds was determined scintigraphically using a Searle Radiographics HP scintillation camera fitted with a pinhole collimator containing a 1-cm diameter hyperbolic aperature. In several cases the dog was sacrificed. Quantitative estimates of organ and tissue distribution of activity were obtained by placing the weighed organs and tissues obtained at necropsy at fixed geometric positions in front of a thallium-activated NaI scintillation detector and analyzing detected scintillation events using routine methods.

Results

A. Synthesis. ^{11}C-Labeled mandelonitrile and *p*-methoxymandelonitrile were prepared from 275 and 417 mCi of K^{11}CN (at t_0) in radiochemical yields of 6 and 5% in a total synthesis time of 50 and 60 min, respectively. ^{11}C-Labeled *p*-hydroxymandelonitrile and 3,4-dihydroxymandelonitrile were prepared from 722 and 827 mCi of K^{11}CN in radiochemical yields of 1 and 3% in a total synthesis time of 59 and 65 min, respectively.

The results obtained in the synthesis of ^{11}C-labeled mandelic acids are listed in Table I. In each case the melting point and infrared spectrum of the product agreed with both that obtained in prior nonradioactive preparations and with the respective data reported in the literature.

^{11}C-Labeled 2,2-dimethyl-5-phenyl-1,3-dioxolan-4-one was prepared from 469 mCi of K^{11}CN via [^{11}C]mandelic acid in a radiochemical yield of 17.5% in a total synthesis time of 63 min: mp 42–43 °C (lit.[13] mp 45 °C). ^{11}C-Labeled ethyl mandelate was prepared in a four-step synthesis from 747 mCi of K^{11}CN via mandelonitrile, mandelic acid, and the dioxolanone in a radiochemical yield of 30% in an overall reaction time of 91 min. ^{11}C-Labeled benzyl mandelate was prepared in an 8.5% radiochemical yield from 925 mCi of K^{11}CN in a total synthesis time of 92 min via [^{11}C]mandelic acid: mp 90.5–92 °C (lit.[4] mp 90–91 °C). The infrared and NMR spectrum of the dioxolanone and esters agreed with that obtained in prior nonradioactive preparations as well as that reported.

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Table I. Preparation of Carbon-11-Labeled Mandelic Acids

$$Ar{-}CHO \xrightarrow[\text{aq } NaHSO_3,\ 0\text{–}5\ ^\circ C,\ 20\ min]{\text{aq } K^{11}CN} Ar{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}{}^{11}CN \xrightarrow[\Delta]{\text{concd HCl}} Ar{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}{}^{11}COOH$$

$K^{11}CN$, mCi, t_0	Ar (5-mmol rxn scale)	Product mCi, t_0	Product mCi, t_{inj}	Radiochemical yield, %	Chemical yield, %	Total synth time, min	Mp, °C
377	C_6H_5	141	16	37	51	65	119–120[a]
519	m-$CH_3C_6H_4$	209	20	40	48	70	90–91[b]
1823	o-ClC_6H_4	502	56	28	31	65	78–80[c]
353	p-ClC_6H_4	88	11	25	49	63	118–119[d]

[a] Lit.[15] mp 119–120 °C. IR spectrum agreed with Sadtler Standard Prism Spectrum No. 13457. [b] Lit.[16] mp 93–94 °C. [c] Lit.[17] mp 84–85 °C. IR spectrum agreed with Sadtler Standard Grating Spectrum No. 13445. [d] Lit.[18] mp 119–120 °C. IR spectrum agreed with Sadtler Standard Prism Spectrum No. 18490.

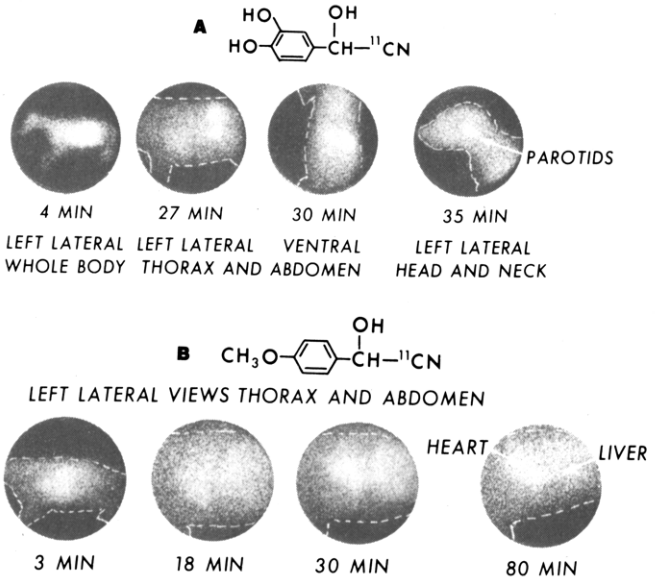

Figure 1. Distribution of ^{11}C activity of 3,4-dihydroxymandelonitrile (A) and p-methoxymandelonitrile (B) in dog. With [^{11}C]-3,4-dihydroxymandelonitrile early images show activity predominantly in the blood pool followed by progressive concentration of activity in the parotids and stomach. Activity is seen with this compound in the region of the heart at 27 min in the left view of the thorax. With [^{11}C]-p-methoxymandelonitrile scintiphotos at 3–5 min show activity predominately in the blood pool followed by progressive concentration of activity in the region of the heart greater than that in the liver in the left lateral views of the thorax at 18, 30, and 80 min.

B. In Vivo Distribution. Serial scintiphotos of ^{11}C activity in the dog following intravenous administration of [^{11}C]-3,4-dihydroxymandelonitrile and [^{11}C]-p-methoxymandelonitrile are shown in Figure 1. The in vivo distribution of [^{11}C]-p-hydroxymandelonitrile is comparable to that of [^{11}C]-3,4-dihydroxymandelonitrile in which scintiphotos at 1–4 min show activity predominately in the blood pool followed by progressive concentration of activity in the parotids and stomach. Greater activity is seen in the region of the heart than in the liver in the left lateral view of the thorax at 27 min. This pattern is perhaps more apparent in the left lateral views of the thorax at 18, 30, and 80 min in the study with [^{11}C]-p-methoxymandelonitrile. Since reduction of [^{11}C]-3,4-dihydroxymandelonitrile yields [^{11}C]norepinephrine, which has been shown to localize in the myocardium,[19] it is intriguing to consider whether reduction of [^{11}C]mandelonitriles can occur in the body resulting in endogenous production and myocardial localization of ^{11}C-labeled neurohumeral amine-like agents.

Serial scintiphotos of ^{11}C activity in the dog following intravenous administration of [^{11}C]-m-methylmandelic acid are shown in Figure 2. Initial distribution of activity in the blood pool is followed by progressive renal excretion with accumulation of activity in the bladder. Comparable results were seen with mandelic acid and with p-chloro- and o-chloromandelic acid.

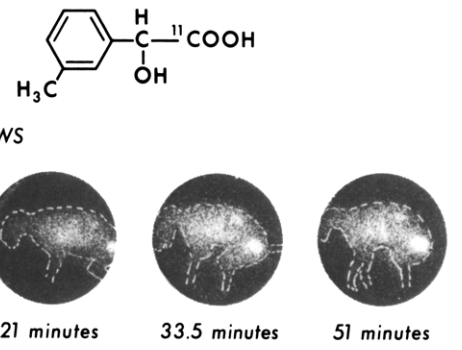

Figure 2. Urinary clearance of ^{11}C activity of m-methylmandelic acid as a function of time. Initial distribution of activity in the blood pool is followed by progressive renal excretion with accumulation of activity in the bladder. Similar distributions were observed with o- and p-chloromandelic acid and mandelic acid itself.

Table II. Comparative Carbon-11 Activity in Tissues Relative to Temporal Muscle Following Intravenous Administration of [^{11}C]-o-Chloromandelic Acid

Organ	(cpm/g of tissue)/(cpm/g of temporal muscle) I (34 min)	II (40 min)
Brain	0.23	0.16
Lungs	1.08	1.60
Heart (washed)	1.15	1.06
Temporal muscle	1.00	1.00
Liver	1.38	1.79
Bile	1.23	0.85
Spleen	1.08	1.46
Stomach	1.35	1.17
Pancreas	4.54	1.18
Kidneys	4.46	6.26
Blood	13.77	
Mesenteric nodes		2.28
Mesenteric fat		0.47
Urine	36.77	295
Adrenal		3.73
CSF	2.00	

Organ distribution of ^{11}C activity in two dogs sacrificed at 34 and 40 min following administration of [^{11}C]-o-chloromandelic acid is shown in Table II. Activity in lungs, heart, liver, bile, spleen, and stomach is more or less comparable. Activity in brain is substantially lower and that in kidneys, blood, and urine substantially higher than activity in other tissues.

The water-insoluble [^{11}C]ethyl and [^{11}C]benzyl mandelates were administered in Me_2SO. Scintiphotos taken within 3 min showed substantial activity retained

in the lungs, presumably related to intravascular precipitation of the agent and mechanical lodging of the precipitate in the pulmonary capillary bed. Subsequent serial scintiphotos showed clearance of activity from the lungs in 5–10 min and slow renal excretion of activity which appeared to be delayed 15–20 min in time relative to the corresponding [^{11}C]mandelic acid.

^{11}C-Labeled 2,2-dimethyl-5-phenyl-1,3-dioxolan-4-one was administered in Me_2SO and was initially retained in the lungs. However, clearance from the lungs was rapid and concentration of ^{11}C activity in the kidneys and bladder was prompt and comparable to that seen with [^{11}C]mandelic acid. Moreover, absorption of expired air from the dog in concentrated $Ba(OH)_2$ showed the presence of $^{11}CO_2$.

Discussion

It has been postulated that certain nitriles such as amygdalin liberate cyanide in vivo and that such regional release of cyanide might be of use in cancer chemotherapy.[1] The scintigraphic evalution of the mandelonitriles supports this notion in that the eventual distribution of [^{11}C]-hydroxymandelonitrile derivatives is similar to that noted with [^{11}C]cyanide,[20] the dissociation product of the [^{11}C]mandelonitriles. It is not clear whether certain nitriles such as *p*-methoxymandelonitrile or mandelonitrile itself can be reduced in vivo to form the corresponding amine. Our results are consistent with but do not establish this possibility. We are not aware of any literature reports describing such a metabolic pathway in animals. However, reduction of nitriles in plants has been suggested.[21,22]

The findings of similar scintigraphic distribution following administration of *m*-methyl-, *p*-chloro-, and *o*-chloromandelic acid and mandelic acid itself suggest that the metabolism of these compounds does not involve aromatic ring hydroxylation, as is the case for many aromatic pharmaceuticals. The –CH(OH)COOH moiety of these mandelic acids appears to determine their renal excretion kinetics, probably through the kidneys' organic acid excretory pathways.

The key role of the free carboxyl group of the mandelic acids is underscored by the delay in renal excretion of ^{11}C activity of the ethyl and benzyl esters. The prompt excretion of activity with the dioxolanone derivative further suggests that hydrolysis of these esters proceeds far more slowly than hydrolysis of the dioxolanone.

It has been reported that phenylglyoxylic acid is a metabolite of mandelic acid.[23] Consequently, the detection of activity in the expired breath of the dog suggests that [^{11}C]mandelic acid produced by in vivo hydrolysis of the [^{11}C]dioxolanone may be undergoing metabolic oxidation to [^{11}C]phenylglyoxylic acid with subsequent decarboxylation to $^{11}CO_2$.

The tissue distribution study performed after administration of *o*-chloromandelic acid showed a disproportionately high blood level of activity suggesting the presence of binding sites for this mandelic acid. The relatively high concentration of activity in the pancreas of one dog and the adrenal of another is unexplained.

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