SYNTHESIS OF 3,5-DIISOPROPYL[CARBOXY-^{14}C]SALICYLIC ACID AND ITS ^{67}CU COMPLEX

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SUMMARY

The synthesis of 3,5-diisopropyl[carboxy-^{14}C]salicylic acid was achieved via Kolbe-Schmitt carboxylation of potassium 2,4-diisopropylphenolate. The yield of this acid was 81% based upon the weight of the product and 93% based upon radioactivity incorporated into the labeled acid which contains 98% ^{14}C in the carboxyl group (specific activity = 5.1 μCi/mg). The labeled acid was characterized by ultraviolet spectrophotometry and purity established by thin-layer chromatography, autoradiography, and liquid scintillation counting. A 90% yield of the double labeled ^{14}C, ^{67}Cu-complex (specific activity = 4.6 μCi ^{67}Cu/mg) was obtained using conditions developed with non-radioactive reactants. The presence of ^{67}Cu in this complex was established using γ-ray emission spectrophometry.

Key Words: 3,5-diisopropyl[carboxy-^{14}C]salicyclic acid, tetrakis-μ-3,5-diisopropyl[carboxy-^{14}C]salicylatodiaquo[^{67}Cu]dicopper(II).

INTRODUCTION

Tetrakis-μ-3,5-diisopropyl[carboxy ^{14}C]salicylatodiaquo[^{67}Cu]copper(II) [$Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_2$] (I) is a binuclear copper complex [1] found to have

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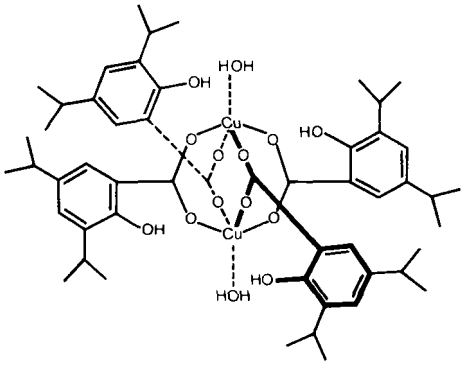
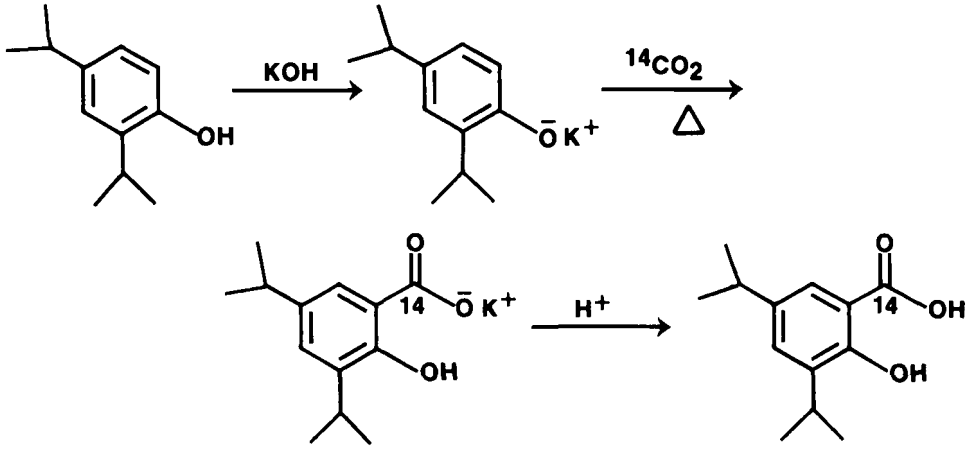
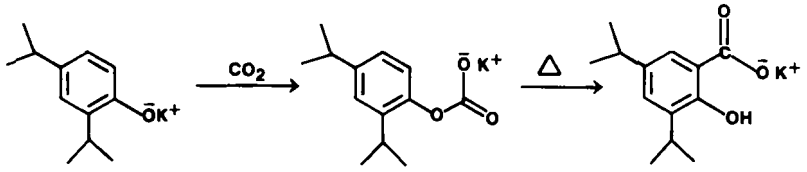
antiinflammatory [2], antiulcer [2], analgesic [3], antidiabetic [4], anticonvulsant [5,6], anticancer [7,8], anticarcinogenic [9], antimutagenic [10], and radioprotectant and radiorecovery activities [11,12] and it is effective in preventing reperfusion injury [13]. These pharmacological activities have been related to its disproportionation of superoxide, the facilitation of de novo synthesis of the copper-dependent and zinc-modulated superoxide dismutase found in all human cells, or facilitation of de novo synthesis of other copper-dependent enzymes required to overcome these disease states [2-13].



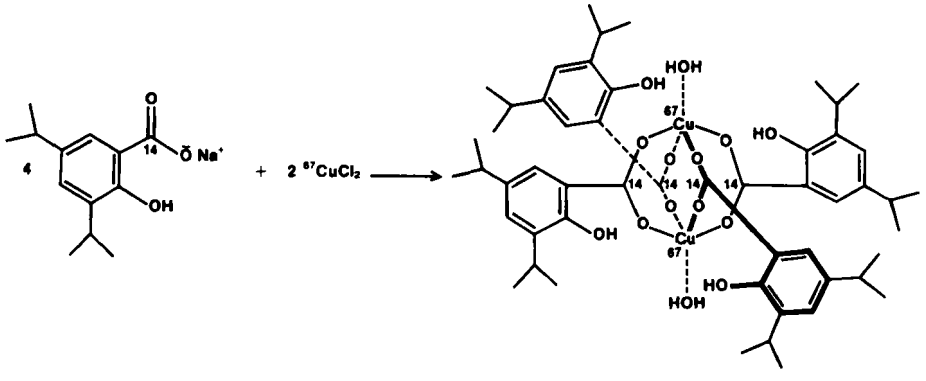
As an approach to understanding the fate of this complex in biological systems it was decided to synthesize the double labeled ^{14}C and ^{67}Cu complex. The first step in achieving this goal was the synthesis of 3,5-diisopropyl[carboxy-^{14}C]salicylic acid using the Kolbe-Schmitt reaction as shown:



The mechanism of the Kolbe-Schmitt reaction has not been definitely established. However, this reaction appears to involve thermal rearrangement of a "carbonate intermediate" formed by the addition of a phenolate to carbon dioxide at room temperature as shown in the following scheme [14-17]:



The ^{67}Cu complex of 3,5-diisopropyl[carboxy-^{14}C]salicylic acid was then synthesized as shown below using established methods [2] giving essentially quantitative yields of well characterized non-radioactive complex [1]. Presence of ^{67}Cu in this complex was demonstrated based upon the γ-ray spectrum of ^{67}Cu.



RESULTS AND DISCUSSION

The required 2,4-diisopropylphenol, which is not commercially available, was synthesized by thermal decarboxylation of 3,5-diisopropylsalicylic acid. Distallation of the reaction product gave a clear colorless liquid which was characterized as the desired phenol by nuclear magnetic resonance and its purity established by elemental analysis. The labeled acid was then synthesized from the anhydrous potassium salt of 2,4-diisopropylphenol with anhydrous (distilled) $^{14}CO_2$ in a closed system.

Highest yields of Kolbe-Schmitt carboxylation product are achieved when the reaction is performed under anhydrous conditions and with anhydrous reagents. The anhydrous potassium salt of 2,4-diisopropylphenol was prepared under nitrogen, which also prevents air oxidation of the phenol, by azeotropic distillation of

water produced following the addition of potassium hydroxide to this phenol in benzene. The isolated anhydrous potassium salt of 2,4-diisopropylphenol was then collected by filtration under nitrogen and stored under vacuum in a vacuum dessicator until it was used in the carboxylation reaction.

Prior to the use of $^{14}CO_2$ to carboxylate potassium 2,4-diisopropylphenol, $^{12}CO_2$ was used to develop reaction conditions that maximized the yield of ^{12}C 3,5-diisopropylsalicylic acid. These reactions were performed at various temperatures and heating times in the closed system used for the ultimate ^{14}C labeling reaction. The optimized synthetic conditions gave the ^{14}C labeled salicylate in excellent yield, 81 percent based upon weight of the product or 93 percent based upon its specific activity, 5.1 μCi/mg.

The 3,5-diisopropyl[carboxy-^{14}C]salicylic acid was identified by ultraviolet spectrophotometry. The ultraviolet spectrum of the ^{14}C labeled acid was essentially identical to the spectrum of the non-labeled acid. Purity of the ^{14}C labeled acid was established by scintillation counting and thin layer chromatography coupled with autoradiography. These procedures revealed a single band that moved parallel to the non-labeled acid.

The reaction of sodium 3,5-diisopropyl[carboxy-^{14}C]salicylate with a mixture of non-radioactive and radioactive copper gave a 90% yield of double labeled ^{14}C, ^{67}Cu-complex containing 0.0006% ^{67}Cu. Specific activity of ^{67}Cu in this complex was 4.6 μCi/mg. The γ-ray emission spectrum obtained for this product contained the expected radiation energies at 184, 93, and 91 KeV for ^{67}Cu.

The availability of this double labeled complex will provide answers to questions concerning the stability and biodistribution of this complex in biological systems. These data will be useful in understanding the remarkable pharmacological effects of $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_2$.

EXPERIMENTAL

Elemental analyses were done by MHW Laboratories, Phoenix, AZ, and agreed with theoretical values within ± 0.4%. Proton NMR spectra were obtained at ambient temperature (25-30°C) with a 60 MHz Varian EM-360 spectrophotometer. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane standard. Ultraviolet spectra were determined in reagent grade

ethanol using Shimadzu-Spectronic 200 UV and Beckman 64 spectrophotometers. Liquid scintillation counting was done with a Packard Tri-Carb 460C Liquid Scintillation System in Universal Scintilator solvent (New England Nuclear). Copper concentrations were measured in 10% HNO_3 using a Instrumentation Laboratory Model IL 157 Atomic Absorption Spectrophotometer. The γ-ray spectrum of ^{67}Cu in the double labeled complex was obtained using a Nuclear Data ND6 Multichannel programmable analyzer (2 Kev per channel) with a 2 x 2 inch NaI detector.

Synthesis of 2,4-Diisopropylphenol: One hundred gms, 0.45 mol, of 3,5-diisopropylsalicylic acid (Aldrich Chemical Company) was placed in a 250 ml three neck round bottom flask with a magnetic bar and fitted with a thermometer, condenser, and nitrogen inlet. The acid was stirred and heated at 195 to 200°C for 17 hours under nitrogen to prevent air oxidation of the phenol formed upon decarboxylation. The phenol was distilled through a Vigreux column under nitrogen at 0.25 to 0.5 mm of Hg and 65 to 75°C. Sixty gms, 75% yield, of colorless phenol was obtained. The distilled phenol ($C_{12}H_{18}O$) gave the expected values for C and H. A carbon-tetrachloride solution of the phenol gave ^1H NMR spectrum with absorptions at δ = 1.10 (6H, singlet, $2CH_3$), 1.19 (6H, singlet, $2CH_3$), 2.74 (1 H, multiplet, $CH(CH_3)_2$, 3.13 (1 H, multiplet, $CH(CH_3)_2$, 5.45 (1 H, singlet, OH), 6.29-6.43 (1 H, 2 singlets, J $\simeq$ 8 Hz, ArH), 6.59-6.76 (2 H, 2 doublets, J $\simeq$ 2 Hz, ArH) and 6.83 (1 H, doublet, J $\simeq$ 2 Hz, ArH). Deuterium exchange with D_2O caused the loss of the phenolic hydrogen resonance at 5.45 ppm.

Synthesis of anhydrous potassium 2,4-diisopropylphenoxide: Potassium hydroxide (Spectrum Chemicals, 85% pure), 1.85 g (0.028 mol) was suspended in 100 ml of benzene in a three neck round bottom flask fitted with a dropping funnel, paddle stirrer, and a nitrogen inlet. Freshly distilled 2,4-diisopropylphenol (5.05g, 0.028 mol) dissolved in 100 ml of benzene was added dropwise under nitrogen while stirring. Upon completion of this addition the dropping funnel was replaced with a Dean-Stark trap and condenser with an attached drying tube filled with Drierite (W. A. Hammond Drierite Company). Water was then removed from the reaction mixture by azeotropic distillation for 2 to 3 hrs. As the distillation progressed the phenoxide dissolved to give a yellow solution. This solution was allowed to cool under nitrogen and filtered into an oven dried round bottom flask under

nitrogen in a plastic glove bag (Aldrich Chemical Company). This flask was then connected to a flash evaporator to remove the solvent. The snow white solid potassium salt was suspended in 50 ml sodium dried hexane and collected by vacuum filtration with a sintered glass filter in a plastic glove bag purged with nitrogen. An additional quantity of sodium dried hexane (50ml) was used to transfer the salt to the filter funnel. The salt was then dried on the filter funnel attached to a laboratory vacuum line (15 mm Hg) in the nitrogen filled glove bag for about an hour. The dried salt, 5.27 gms (87% yield), was weighed in the nitrogen filled glove bag and transferred to a vacuum dessicator charged with anhydrous phosphorous pentoxide and evacuated (5 to 10 μm Hg) while in the nitrogen atmosphere.

Synthesis of 3,5-diisopropyl[carboxy-^{14}C]salicylic acid: The apparatus used for the ^{14}C Kolbe-Schmitt carboxylation is shown in Figure 1.

One and one-fourth mCi of $^{14}CO_2$ (± 10%, with a specific activity of 1 mCi per m mol, 98% $^{14}CO_2$) was purchased from New England Nuclear in a break-sealed round bottom glass flask with a 18/9 outer ball-socket joint. The ball-socket joints provide safety in allowing flexibility at the connection. A 3 mm long and 0.5 mm diameter cylindrical bar magnet was gently inserted into the open end of the flask and allowed to rest on the break-seal. Surfaces of the ball-socket joint were lubricated with silicone grease and the flask attached with a ball-socket joint clamp to an inner ball-socket joint on the gas-bridge mounted on ring stands in a fume hood (Figure 1A). The attached flask was then cooled with a Dewar containing liquid nitrogen and evacuated (5 to 25 lm Hg) slowly to avoid movement of the bar magnet and breakage of the seal.

The reaction tube was charged with a 2-fold excess of potassium 2,4-diisopropylphenol so that the limiting reagent was the $^{14}CO_2$ and prepared for attachment to the gas-bridge under nitrogen in a plastic glove bag. An oven dried 30 cm long 11 mm internal diameter thick walled glass gas reaction tube fitted with a 18/9 outer ball-socket joint was charged with 10 to 15 oven dried 3 mm glass beads, 545 mg (2.5 mmol) of potassium 2,4-diisopropylphenolate, and covered with an additional 10 to 20 glass beads. The ball joint was then lubricated with silicone grease, stoppered with a No. 3 rubber stopper, and the contents mixed with a Vortex-Gennie stirrer (Fischer Scientific) to intersperse the potassium phenolate

and glass beads. The charged reaction tube was then attached to the gas-bridge with a ball-socket clamp, evacuated (5 to 25 μm Hg), and cooled with a Dewar of liquid nitrogen (Figure 1A).

After evacuation, closure of the evacuation outlet, cooling of the reaction tube, and opening of the gas-bridge to the reaction tube the break-seal was broken by raising the magnet bar and allowing it to fall on the seal. After breaking the seal the Dewar was removed from the $^{14}CO_2$ flask and replaced by a beaker of warm tap water to distill the $^{14}CO_2$ into the liquid nitrogen cooled reaction tube. Following complete distillation (30 minutes) the reaction tube was sealed by heating it 2 inches below the ball-socket joint with a hand held propane torch. The reaction tube can be twisted as the glass softens to effect sealing, however, a large volume of melted glass should be used to evidence sealing. After the sealed tube cooled at the sealed end the tube was removed from the Dewar and contents mixed using a Vortex-Gennie stirrer for 30 minutes. (Since the $^{14}CO_2$ is the limiting reagent and it rapidly reacts with the potassium phenolate the pressure inside the tube is less than atmospheric pressure).

Following mixing and carbonate formation the sealed tube was heated in a sand bath at 150 to 160°C for 10 hrs to effect rearrangement of the carbonate. At regular intervals, every 3 to 4 hrs, contents of the hot reaction tube were mixed (asbestos glove). After heating for 10 hrs the reaction product had the appearance of a tan colored powder mixed with red oil.

Sometime prior to the end of the thermal rearrangement step, 5 ml of a saturated aqueous solution of barium hydroxide and a Teflon coated stirring bar were placed in a 50 ml round bottom flask which was connected to the gas-bridge (Figure 1B), frozen with liquid nitrogen, and evacuated (5 to 25 μm Hg).

Upon completion of the thermal rearrangement the reaction tube is allowed to cool to room temperature, etched with a circular glass cutter just below the seal, and then the lower half of the tube cooled in a Dewar of liquid nitrogen. The seal was then heated above the etch with the hand held propane torch and drops of water applied to the etch with a disposable Pastuer pipette to cause a crack along the etch mark and enable removal of the sealed portion of the reaction tube. A pliers and file may be used to remove the cracked seal. The open reaction tube is then placed in a liquid nitrogen cooled 30 cm long and 20 mm internal diameter

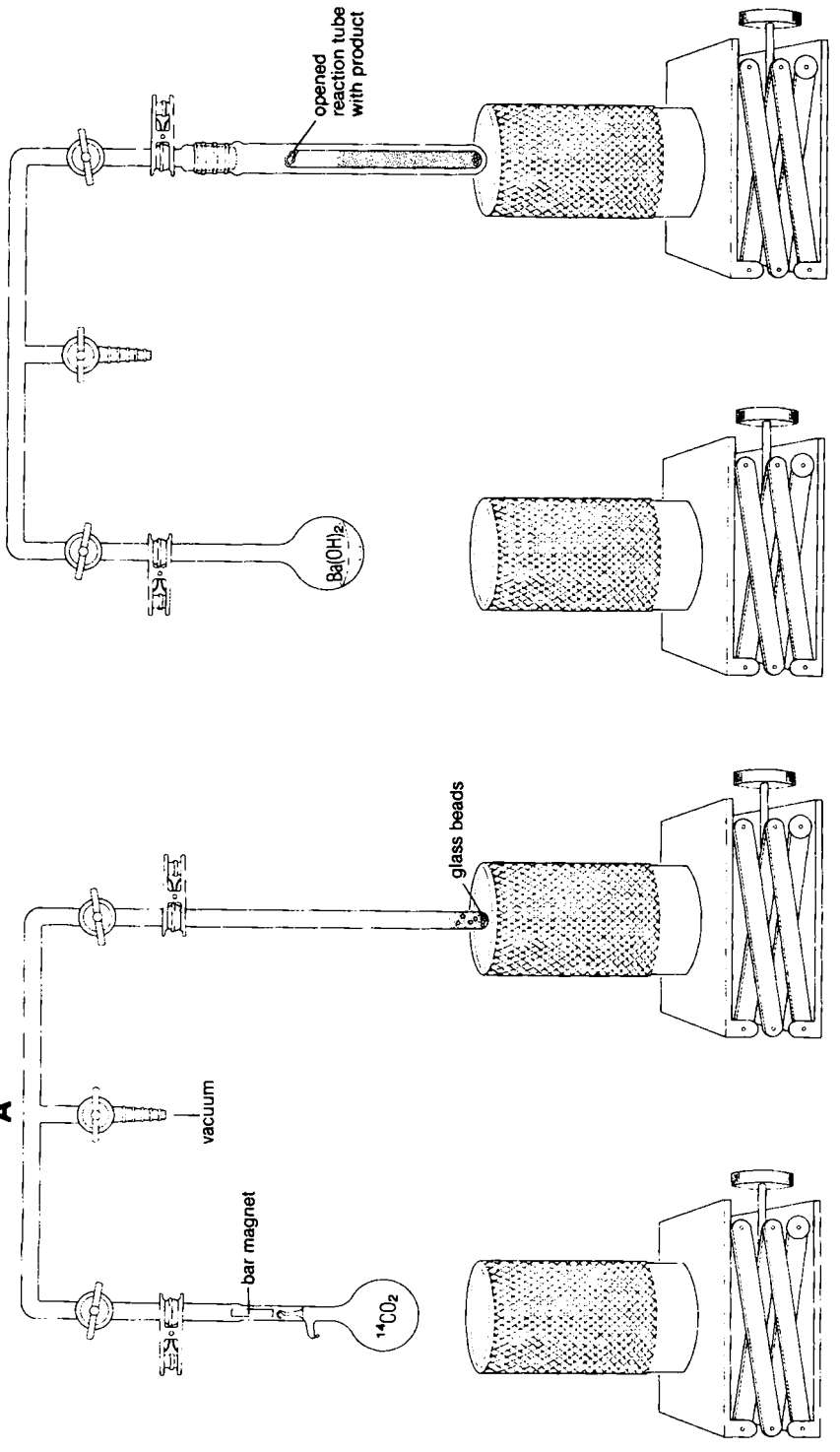


Figure 1. Apparatus for the distillation of $^{14}CO_2$ into flask containing the potassium salt of the phenol (A) and for the recovery of non-reacted $^{14}CO_2$ (B).

dessicator tube with a 29/42 standard taper opening and placed in the Dewar of liquid nitrogen. An 18/9 ball-socket and 29/42 standard taper adapter is then attached to the dessicator tube and this assembly attached to the gas-bridge and evacuated (Figure 1B). The Dewar of liquid nitrogen was then removed from the dessicator which was allowed to warm to room temperature causing the non-reacted $^{14}CO_2$ to distill into the liquid nitrogen cooled flask containing the frozen solution of barium hydroxide. After distillation (30 minutes) the gas-bridge stopcock above the barium hydroxide was closed, the barium hydroxide solution allowed to warm to room temperature with the aid of a warm tap water bath, and the solution stirred for 3 hrs. The radioactive $^{14}CO_2$ collected in this trap was then disposed of in an appropriate manner. Only background radiation was measured in this trap and the barium hydroxide was flushed down an approved radioactive waste disposal sink. The reaction tube was then removed from the dessicator and the 3,5-diisopropyl[carboxy-^{14}C]salicylic acid isolated and characterized.

The crude reaction product was transferred to a separatory funnel with 50 ml 5% $NaHCO_3$ and 30 ml n-hexane. After separation the hexane layer was again extracted with 50 ml of 5% $NaHCO_3$. The combined $NaHCO_3$ extracts were acidified (pH 1.0) with concentrated HCl and left to stand for 12 hrs. The precipitate was filtered and dried on the filter funnel at laboratory vacuum (15 mm Hg) for 18 hrs. Yield of the labeled acid was 226 mg, 81% of theory (280 mg). Yield based upon the total incorporated radioactivity, 1.162 m Ci (Table I), is 1.162 m Ci/1.25 m Ci (theory) or 93%.

Characterization of 3,5-diisopropyl[carboxy-^{14}C]salicyclic acid: The ^{14}C labeled reaction product was characterized as 3,5-diisopropyl[carboxy ^{14}C]salicylic acid based upon a comparison of its ultraviolet spectrum with a spectrum obtained for an authentic sample of 3,5-diisopropylsalicylic acid. These spectra are presented in Figure 2

Three maxima for the authentic sample of 3,5-diisopropylsalicylic acid were observed at 314 nm, 240 nm, and 210 nm and these absorptions corresponded with values of 314 nm, 240 nm, and 208 nm for the ^{14}C labeled acid, confirming the structural assignment. The regression equation for a Beer-Lambert plot of absorbance at 314 nm versus 5 concentrations of the authentic sample of 3,5-diisopropylsalicylic acid was used to estimate the concentration of solutions of

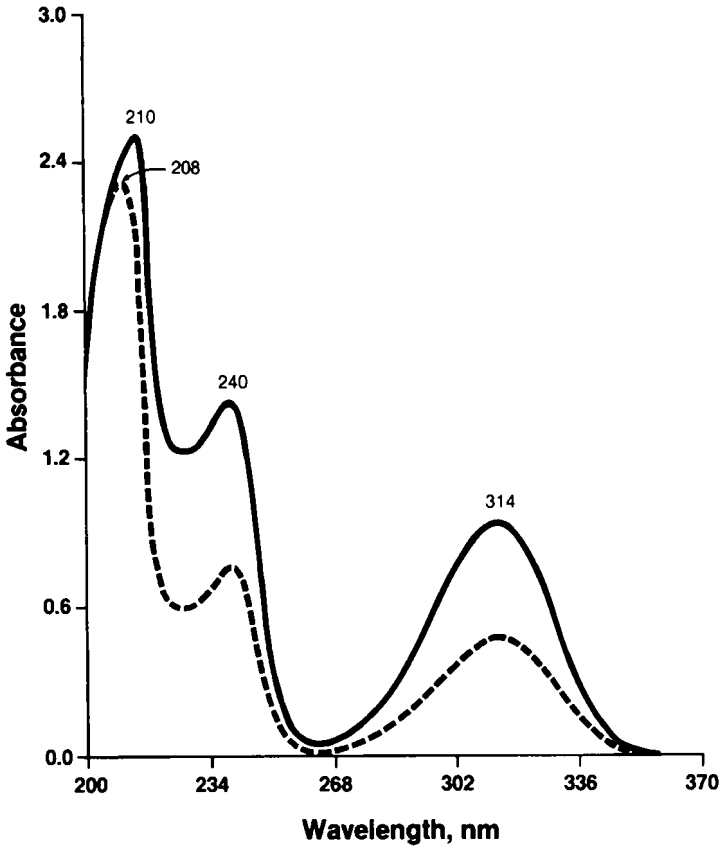


Figure 2. Ultraviolet spectra of 3,5-diisopropylsalicylic acid (----), molar absorptivity = 4118 $M^{-1}cm^{-1}$, and 3,5-diisopropyl[carboxy-C^{14}] acid, (——), estimated molar absorptivity = 4213 $M^{-1}cm^{-1}$, in 100% ethanol.

the labeled acid in calculating the incorporation of radioactivity (Table 1) and the radioactivity of these samples was determined using liquid scintillation.

Thin layer chromatograms of an authentic sample of 3,5-diisopropylsalicylic acid and the isolated ^{14}C labeled acid were run in parallel to further characterize the product and demonstrate its purity. A sample of the authentic acid and the isolated reaction product were chromatographed on 60 F_{254} silica gel plates (Merck and Company) using a 1.8:10:8 acetic acid:benzene:n-hexane mobile phase. The position of the authentic sample of 3,5-diisopropylsalicylic acid (A) was located with a 2% aqueous $FeCl_3$ (Analytab Products) spray and the position of the ^{14}C labeled acid (B) was located by an autoradiographic 5 day exposure to x-ray film. Results presented in Figure 3 show that both compounds moved at the same rate and share this physical-chemical property and both compounds are pure by

TABLE I. 3,5-Diisopropyl[carboxy-^{14}C]salicylic acid specific activity determination

A. Counting Efficiency:

^{14}C Toluene[a] (μL)	Observed[b] (cpm)	Mean cpm minus[c] background	Activity[a] (dpm)	%Counting Efficiency (mean cpm/dpm x 100)
25	9662			
25	9540	9643	11,750	82
25	9780			

[a] = NBS standard, 4.7×10^4 dpm/100 μL ± 3.8%, [b] = Average of duplicate determinations, [c] = Mean background = 18 cpm

B. Specific Activity:

^{14}C-acid[a] Solution (μL)	Observed[b] (cpm)	Mean cpm minus Background	Total radioactivity[c] (nCi)	Total quantity (g); based on $A_{314\ nm}$[d]	Specific Activity[e] nCi/ g	Total Radioactivity Incorporated in 226 mg product (mCi)[f]
25	2292	2274	1.25	0.2527	4.947	1.118
50	4783	4765	2.62	0.5055	5.183	1.171
100	9774	9756	5.36	1.0109	5.302	1.198
						1.162 mCi (mean)

[a]3,5-diisopropyl[carboxy-^{14}C]salicylic acid, [b]mean of two samples in duplicate, [c]calculated by the relation:

$$\text{counting efficiency (82\%)} \times 2.2 \times 10^3 \text{ dpm/nCi}, \quad \frac{\text{mean cpm}}{}$$

[d]calculated by the linear regression equation: $y = -1.678 \times 10^3 + 1.896 \times 10^2 x$, where y = absorbance at 314 nm and x = total concentration in μg, correlation coefficient r = 0/999. [e]Total radioactivity/Total quantity, [f]nCi/ g x 226 mg

these measurements. The optical density scan (C) of the autoradiograph, which also indicates the origin (bottom) and solvent front (top) of both chromatograms, provides additional evidence for the identity and purity of the isolated ^{14}C labeled reaction product.

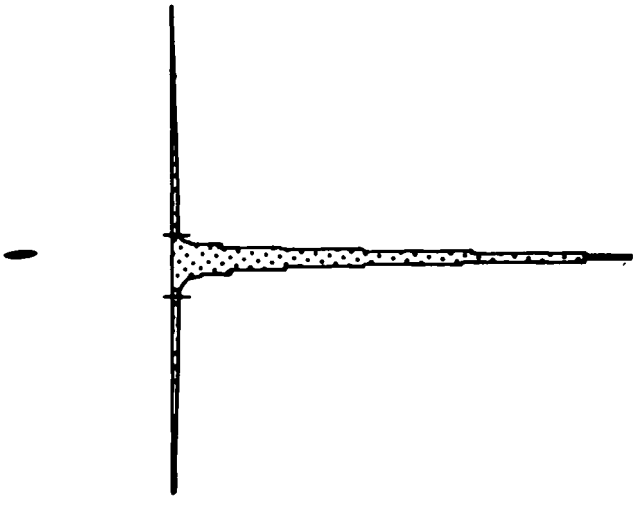


Figure 3. Thin layer chromatograph of an authentic sample of 3,5-diisopropylsalicylic acid developed with 2% aqueous $FeCl_3$ spray (A), autoradiograph of the thin layer chromatogram of ^{14}C labeled acid run parallel to a sample of authentic acid (B), and optical density scan of the autoradiograph (C).

Studies of reaction conditions on yield of $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_2$: Synthetic conditions reported for the synthesis of this complex [2] were studied to optimize the yield of $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_2$. These studies are summarized in Table 2. Varying amounts of the 3,5-diisopropylsalicylic acid (16.5 mg to 1652 mg; 0.0742 mmol to 7.43 mmol) were dissolved in a separatory funnel using various volumes of deionized distilled water and the required volume of 10% NaOH. These clear solutions were then transferred to a beaker and titrated to various pH values (7.01 to 9.05) using 10% or 1% HCL. Non-Radioactive reagent grade (Spectrum Chemicals) $CuCl_2(H_2O)_2$ (6.3 mg to 510 mg; 0.037 mmol to 3.77 mmol) was dissolved in 1 ml of deionized distilled water (pH 3.99) and dropped into these solutions of sodium 3,5-diisopropylsalicylate. The tan colored precipitates obtained were stirred for one hour and filtered using a sintered glass filter funnel. The pH values of the resultant filtrates ranged from 4.38 to 5.74. These precipitates were washed with a large excess of water (20 to 100 ml) and air dried using a

laboratory vacuum line (15 mm Hg). After passsing air through the funnel for 2 hours the dried preparation was found to be analytically pure $Cu(II)_2$(3,5-diisopropylsalicylate)$_4(H_2O)_2$. Anal. $Cu_2C_{52}H_{58}O_{12}$, Cu,C,H. The isolated complex has been characterized as a binuclear complex using spectrophotometric methods [1].

A typical example of the progressive drying of this copper complex in a filter funnel is shown in Figure 4. The filtrate was concentrated and the precipitate collected by filtration, dried, and weighed to determine the amount of water soluble complex shown in Table 2.

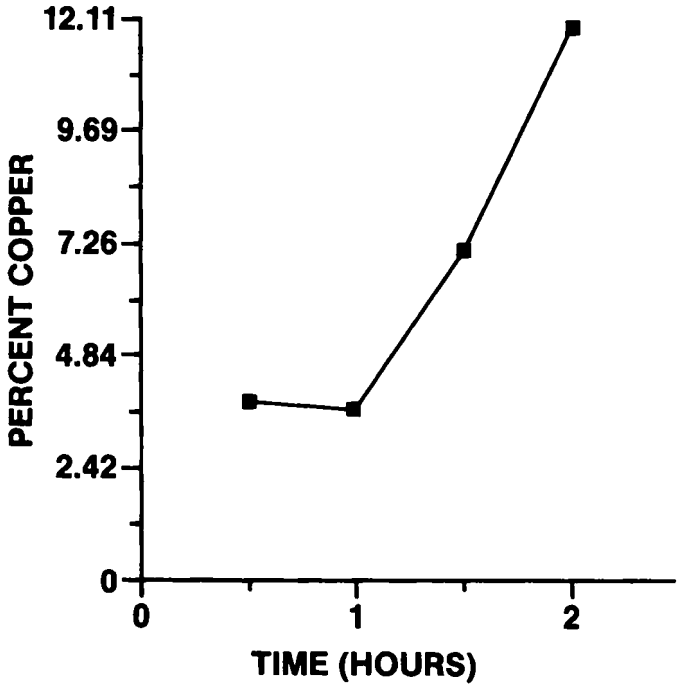


Figure 4. Time dependent change in copper content found for the water-wet $Cu(II)_2$(3,5-DIPS)$_4(H_2O)_2$ (12.11% Cu) precipitate on drying in a sintered glass filter funnel attached to a laboratory vacuum line (15 mm Hg).

Maximal yields were obtained with concentrated solutions of these reactants wherein the pH of the ligand solution ranged from 7 to 9. Microcentrifugation is recommended for the isolation of smaller amounts (10 to 20 mg) of complex.

Preparation of $^{67}Cu(II)_2$(3,5-Diisopropyl[Carboxy-^{14}C]Salicylate)$_4(H_2O)_2$: The 3,5-diisopropyl[carboxy-^{14}C]salicylic acid (422 μCi, 82.5 mg; 0.371 mmol) was

TABLE 2. Synthetic conditions studied to optimize the yield of $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_2$ (M.W. 1048.48 Daltons)

3,5-DIPS* (mmol)	Volume of H_2O (ml)	pH	$CuCl_2(H_2O)_2$** (mmol)	Volume of H_2O (ml)	pH	Final pH	Theoretical Yield (mmol)	Experimental Yield (%)	Water Soluble (%)	Total (%)
7.43	200	9.05	3.77***	30	3.94	4.38	1.85	75	n.d.	--
0.74	20	9.10	0.37	5	3.99	4.47	0.19	52	n.d.	--
0.74	20	6.99	0.37	5	3.99	4.93	0.19	72	11	83
1.49	30	6.98	0.74	5	3.99	4.93	0.37	72	16	88
0.74	10	7.01	0.37	1	3.94	4.64	0.19	93	2	95
0.74	15	7.02	0.37	1	3.99	5.74	0.19	87	10	99
0.74	10	7.06	0.37	1	3.99	5.25	0.19	86	11	97
0.74	8	7.01	0.37	1	3.99	4.50	0.19	91++	0	91
0.74	8	9.03	0.37	1	3.99	4.67	0.19	89++	7	95
0.74	10	8.05	0.37	1	3.99	4.68	0.19	88++	7	95
0.37	10	8.00	0.185	1	3.99	4.97	0.093	83	n.d.	--
0.18	10	8.25	0.0927	1	3.99	5.02	0.046	47	n.d.	--
0.07	10	7.42	0.037	1	3.99	4.56	0.019	46+	n.d.	--

*3,5-diisopropylsalicylic acid; 222.28 g/mol; **$CuCl_2(H_2O)_2$; 170.48 g/mol; ***Anhydrous $CuCl_2$ (2% excess); 135.45 g/mol; n.d. - not determined; +collected on filter paper; ++Anal. $Cu_2C_{52}H_{68}O_{12}$, C, H.

dissolved in 5 ml of deionized water with 0.35 ml of 10% NaOH in a 30 ml separatory funnel. This clear solution (pH 12.33) was transferred to a 20 ml beaker with a 1 ml deionized water wash and titrated to pH 8.05 (pH meter) using 10% or 1% HCl.

Non-Radioactive reagent grade $CuCl_2(H_2O)_2$ (31.5 mg; 0.185 mmol) was dissolved in 1 ml of deionized water and mixed with $^{67}CuCl_2$ (1.0 mCi, 0.2 μg; specific activity 16.5 mCi/μg) dissolved in 0.02 ml of 2.0 M HCl as supplied by Los Alamos Laboratory and dropped into the solution of sodium 3,5-diisopropyl[carboxy^{14}C]-salicylate over a period of 2 minutes with rapid stirring, using a micro Teflon coated stirring bar. The tan colored precipitate was stirred for about one hour before collection by filtration using a 15 ml sintered glass filter funnel. The pH of the filtrate was 4.79. The precipitate was washed with 20 ml of deionized water and air dried (5 hrs) using a laboratory vacuum line (15 mm of Hg). The dried precipitate weighed 87 mg, a 90% yield (98% ^{14}C and 0.0006% ^{67}Cu). The γ-ray spectrum (Figure 5) shows the expected peaks for ^{67}Cu at 184 KeV, with a 48% abundance obtained by integration between the arrows, 93 KeV, and 91 KeV.

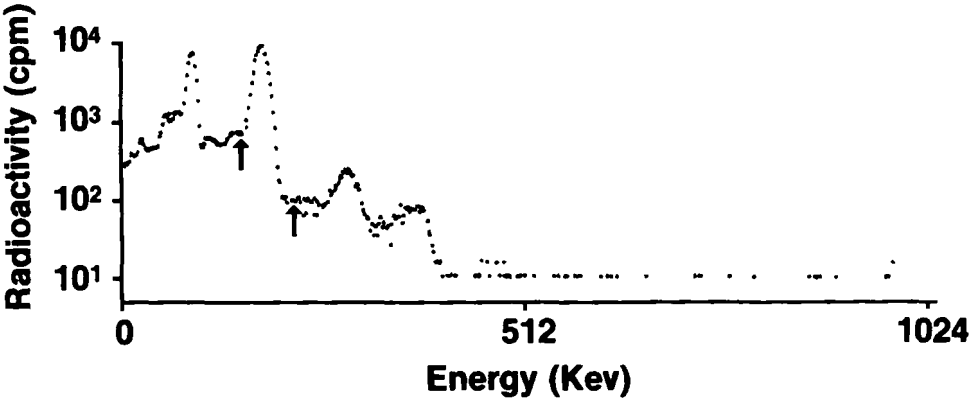


Figure 5. The γ-ray spectrum of the ^{67}Cu labeled complex. The major photopeak at 184 KeV is located between the two arrows.

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