weight of chicks at 8 weeks of age for rations A and B was significantly higher ($P < 0.05$) than those of A_1 and B_1, respectively. The available lysine values when estimated by the present chemical method were also higher in rations A and B. The average gains in weight of the chicks fed with rations C, C_1, C_2, C_3, and C_4 were also significantly different ($P < 0.05$). It was observed from the furnished data that the gain in weight of the chicks increased with the increase of lysine availability. The results showed that the chemically estimated values conformed well with the biologically determined values and thus the present method was very useful for the assessment of compounded poultry rations. Moreover, the present colorimetric method is to some extent more rapid than the method of Rao et al. (1963) because it would then not be necessary to separate dinitrophenylated and free amino acids by ion exchange column chromatography for the estimation of available lysine in the compounded rations.

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Synthesis and Mass Spectrometry of Isotopically Labeled Isopropyl 2-(4-Thiazolyl)-5-benzimidazolecarbamate (Cambendazole)

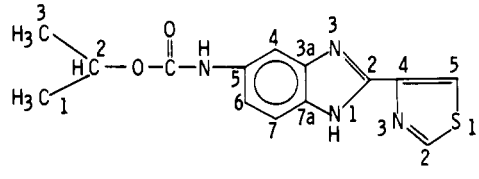
Robert L. Ellsworth,* Holly E. Mertel, and William J. A. VandenHeuvel

The synthesis of a number of heavy atom and radioactively labeled cambendazoles [isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate] for use in metabolism and residue studies is described. Availability of the former has facilitated study of the mass spectrometric fragmentation of this drug. The feasibility of establishing specific activities of the ^{14}C-labeled cambendazoles by mass spectrometry has been demonstrated.

Metabolism and tissue residue studies are integral components in the development of a new animal health drug for use in food-producing animals. Such investigations are facilitated by the use of isotopically labeled drugs, usually with radioactive, but occasionally with stable or heavy isotopes. The former are indispensable for determining levels of drug-related material in tissue, while the latter can be helpful in establishing the structure of metabolites and their mechanism of formation. We have recently prepared cambendazole, isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate, an anthelmintic agent (Hoff et al., 1970), with both stable and radioactive labels in a variety of positions. The compounds discussed in this report are presented in Table I.

Cambendazole in which the thiazole ring nitrogen is enriched with ^{15}N was used in metabolic studies to determine the source of the nitrogen atom (body pool or thiazole N) in 2-carboxamido-5-isopropoxycarbonylaminobenzimidazole (Ib), a urinary metabolite of the drug (VandenHeuvel et al., 1974). Cambendazole enriched with ^{13}C in the C-2 (imidazole ring) position was used in an in vitro metabolism study (Wolf et al., 1974). A number of radiolabeled species of cambendazole have been prepared for use in tissue residue studies to partially characterize the nature of the residue. The availability of the heavy atom labeled cambendazoles has facilitated study of the mass spectrometric fragmentation of this drug. Furthermore, we have examined several of the ^{14}C-labeled preparations by mass spectrometry to determine the role this technique might play in establishing specific activities of labeled compounds independent of radioactivity measurements.

Table I. Isotopically Labeled Cambendazoles



Label type	Label position	% enrichment or sp act.
^{13}C	Thiazole C-4	57%
^{15}N	Thiazole N-3	33%
^{14}C	Benzimidazole C-2	2.81 mCi/mmol
^{14}C	Benzimidazole C-3a,4, 5,6,7,7a (*ar-U-*^{14}C)	2.84 mCi/mmol 9.00 mCi/mmol 114.16 mCi/mmol
^{14}C	Carbamoyl C=O	1.93 mCi/mmol

RESULTS AND DISCUSSION

The radioactive starting material for the compounds labeled in the benzenoid ring with carbon-14 was aniline-^{14}C. The process (Schmid et al., 1966) used by our supplier to prepare this substance from barium carbonate-^{14}C consisted of reduction to barium carbide-^{14}C,

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065.

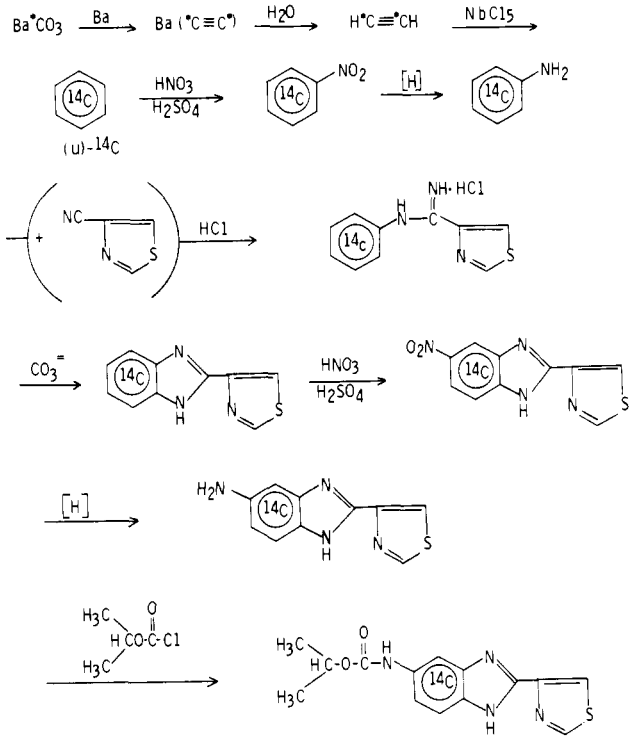


Figure 1. Reaction sequence for benzene ring labeled cambendazole.

liberation of acetylene-^{14}C, dilution with carrier acetylene, and oligomerization to benzene-^{14}C over niobium pentachloride, followed by nitration and reduction (see Figure 1).

Thiabendazole-*ar*-*U*-^{14}C (where *ar* represents the benzene ring) was derived from aniline-^{14}C hydrochloride via the amidine resulting upon hydrogen chloride catalyzed addition to 4-cyanothiazole (Jones and Gal, 1967; Davis et al., 1964) (see Figure 1). Treatment of the amidine with aqueous sodium hypochlorite, followed by basification and warming, led to 2-(4-thiazolyl)benzimidazole-*ar*-*U*-^{14}C, Thibenzole (Grenda et al., 1965). Nitration, reduction, and treatment with isopropyl chloroformate yielded isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate-*ar*-*U*-^{14}C, cambendazole (Hoff and Fisher, 1970).

A sample of glycine-^{15}N served as starting material for the nitrogen-15 labeled compound. Fisher esterification, N-formylation, and C-formylation, followed by reaction with phosphorus pentasulfide, gave 4-thiazole-^{15}N-carboxylic acid (see Figure 2) (Tocco et al., 1964). The acid chloride derived from this acid reacted with isopropyl 4-amino-3-nitrocarbanilate (Ellsworth et al., 1971) to give isopropyl 3-nitro-4-(4-thiazole-^{15}N-carboxamido)carbanilate. This substance, upon catalytic reduction and gentle warming, cyclized smoothly to isopropyl 2-(4-thiazolyl-^{15}N)-5-benzimidazolecarbamate, cambendazole-*thiazole*-^{15}N.

A process exactly similar to that just described, using glycine-2-^{13}C, gave isopropyl 2-(4-thiazolyl-*4*-^{13}C)-5-benzimidazolecarbamate, cambendazole-*thiazole-4*-^{13}C. In the same manner isopropyl-2-(4-thiazolyl)-5-benzimidazole-2-^{14}C-carbamate, cambendazole-*imidazole*-2-^{14}C, was derived from glycine-*1*-^{14}C.

Labeling in the carbonyl group of the carbamate moiety was achieved by reaction of 5-amino-2-(4-thiazolyl)-benzimidazole with isopropyl chloroformate-^{14}C derived from isopropyl alcohol and phosgene-^{14}C.

The mass spectrometric behavior of cambendazole is dominated by fragmentations involving the isopropoxycarbonylamino side chain (Figure 3) (VandenHeuvel et al., 1972). Although the molecular ion is intense, the base peak is found at *m/e* 260, M − 42, and arises via a McLafferty rearrangement involving loss of C_3H_6 from the side chain; a metastable peak for this elimination is found at *m/e* 224. Other intense ions are found at *m/e* 216, M − (42 + 44), and *m/e* 215, M − (42 + 45), resulting from loss of C_3H_6 and CO_2 or CO_2H, respectively. A metastable peak is found at *m/e* 180.1 for the 260 → 216 transition. The mass spectra of the ^{13}C- and ^{15}N-labeled cambendazoles confirm that these fragmentations do not involve the thiazole C-4 or N atoms. The same is true for the ions of *m/e* 242 and 243; these must then be losses of the elements of 2-propanol and C_3H_7O, respectively. In contrast, the ion of *m/e* 189 retains the 4-^{13}C, but not the ^{15}N label and thus it is M − (42 + 44 + 27), involving loss of HCN from the thiazole ring. A metastable peak for the transition 216 → 189 is found at *m/e* 165. The ion of *m/e* 111 retains both

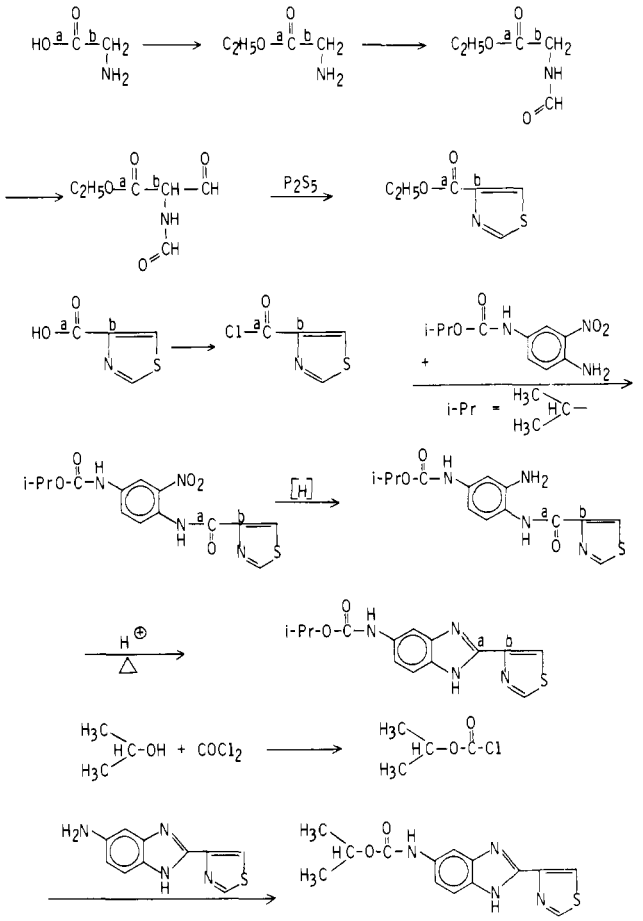


Figure 2. Reaction sequence for other labeled cambendazoles.

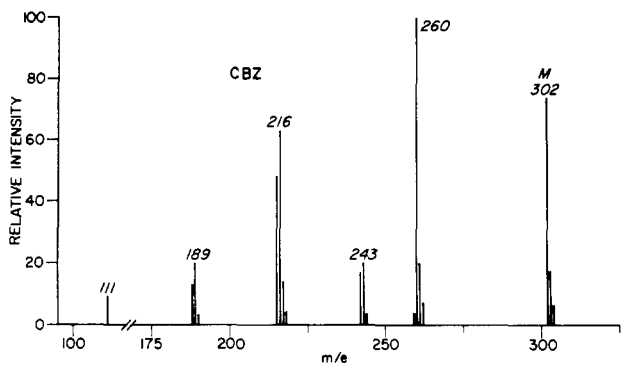


Figure 3. Mass spectrum of cambendazole.

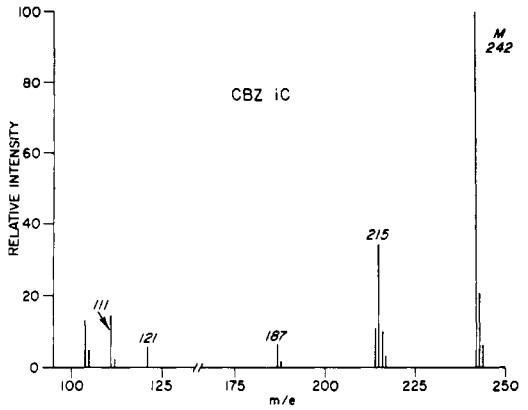


Figure 4. Mass spectrum of the isocyanate resulting from thermal elimination of 2-propanol from cambendazole.

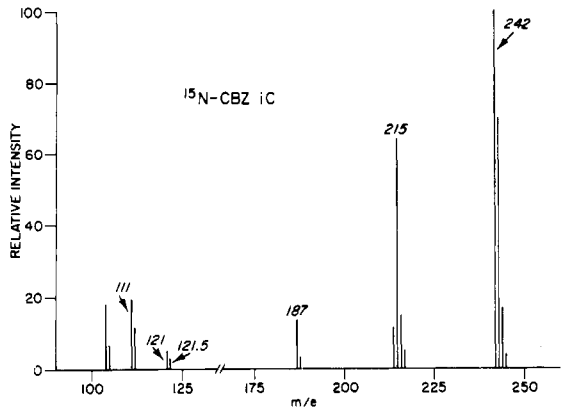
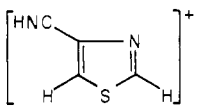


Figure 5. Mass spectrum of the isocyanate resulting from thermal elimination of 2-propanol from cambendazole-*thiazole*-^{15}N.

labels, and a likely structure is then:



When subjected to GLC–MS at temperatures >220 °C cambendazole undergoes an "on-column" thermal loss of 2-propanol to yield the corresponding isocyanate (VandenHeuvel et al., 1972). The major fragment ion of this compound is found at m/e 215 (Figure 4), and has been ascribed to loss of HCN. This proposal has been confirmed by combined GLC–MS of the ^{15}N-thiazole-labeled cambendazole. The mass spectrum of the resulting isocyanate is presented in Figure 5. Enhancement of the isotope peak (m/e 243) of the molecular ion (m/e 242) (compare Figures 4 and 5) is not present with the M – 27 ion (whereas the label is retained with the ^{13}C-labeled isocyanate from cambendazole-^{13}C). The ion of m/e 187 in the isocyanate also retains the ^{13}C label, but not the ^{15}N label, and probably is M – (27 + 28), loss of HCN and CO (from the isocyanate group). The signal at m/e 121 arises from the doubly charged molecular ion; note the isotope peak at the nonintegral mass, 121.5. The spectra of the isocyanate also contain the thiazole-containing fragment ion of m/e 111 described earlier.

The mass spectra of ^{14}C-labeled compounds of low specific activity are not discernibly different from those of the unlabeled species. At considerably increased levels of radioactivity (e.g., 3–10 mCi/mmol) the contribution of the ^{14}C isotope becomes apparent. These and higher specific activity compounds can be employed to study fragmentation patterns and metabolic–biosynthetic

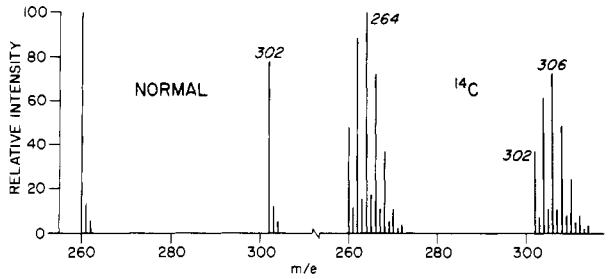


Figure 6. Partial mass spectra of cambendazole (left panel) and cambendazole-ar-*U*-^{14}C of high specific activity (right panel).

pathways in the same way as ^{13}C-labeled compounds. Knoppel and Beyrich (1968) collected ions from ^{14}C-labeled benzene on a graphite plate and used autoradiography to ascertain which ions contained the ^{14}C label. Occolowitz (1968) studied ^{14}C as a label with scanning mass spectrometry, and calculated the ^{14}C compositions of ions. White and co-workers (1972) reported on the use of a ^{14}C label to characterize a fragment ion from the bis(trimethylsilyl) derivatives of adenine, and Goldstein and Rainey (1973) have recently discussed specific activity measurements of this and other ^{14}C-labeled purines by mass spectrometry. Mass spectrometric techniques have been employed by Chalmers et al. (1971) to demonstrate the presence of the ^{14}C label in the catechol moiety of 3-*O*-^{14}C-methyl-4-hydroxyphenylalanine prepared by incubation of L-dopa with methyl-^{14}C-*S*-adenosylmethionine in the presence of catechol-*O*-methyltransferase. Walker et al. (1972) used the same methyl donor to demonstrate the enzymatic conversion of *N*-methyltryptamine to *N*,*N*-dimethyltryptamine by indoleamine-*N*-methyltransferase; the mass spectrum of the in vitro product contained dimethylaminomethylene fragment ions from the side chain at both m/e 58 and 60.

Several forms of ^{14}C-labeled cambendazole were investigated by mass spectrometric techniques, including C-2 labeled (Table I, 3) and benzene ring labeled (Table I, 4).

The specific activities of these compounds were determined by an approach involving calculation of the "weight average molecular weight" (M_w) (VandenHeuvel et al., 1973) from the molecular ion clusters of the labeled and unlabeled species using the expression:

$$M_w = \Sigma mI/\Sigma I$$

where m is the m/e value and I is the corresponding ion intensity (average of three scans) relative to that of the molecular ion. The net label is obtained from these values, and the ^{14}C content then determined. (Our approach to the mass spectrometric determination of specific activity differs from that of Bowen et al. (1972); these authors employ only the intensities of the M, M + 2, M + 4, etc., ions in their direct calculations.) For example, ring-labeled cambendazole from aniline with a very high specific activity was found to possess a M_w of 305.85, compared to 302.25 for the unlabeled drug. The net label is thus 3.6. The most intense signal in the molecular ion cluster from this labeled cambendazole is M + 4, m/e 306, and ions of this sample containing from none to six atoms of ^{14}C (m/e 314) can be discerned in the spectrum (Figure 6). The fragment ion M – 42 does not involve the loss of benzene ring carbon atoms; the fragment ion isotope pattern mirrors that of the molecular ion. On the average, each molecule of labeled compound is 3.6 mass units heavier than the unlabeled compound, and thus on the average each molecule of labeled drug contains 1.8 atoms of ^{14}C. As the specific activity per atom of pure ^{14}C is 62.4

Table II

Labeled cambendazole	Sp act., mCi/mmol MS	LSC
Ring-^{14}C (high)	118	114
Ring-^{14}C (low)	9.4	9.0
C-2-^{14}C	2.6	2.8

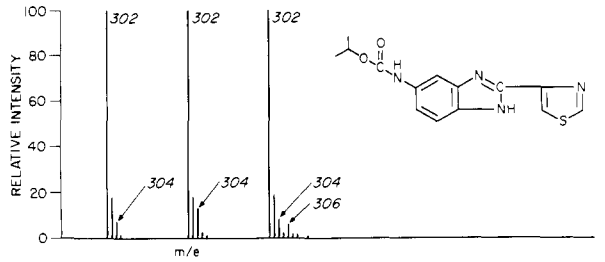


Figure 7. Molecular ion regions from mass spectra of cambendazole (left), cambendazole-*imidazole-2-*^{14}C (middle), and cambendazole-*ar-U-*^{14}C of low specific activity (right).

mCi/mmol, the mass spectrometrically determined specific activity of this particular cambendazole-^{14}C is 118 mCi/mmol (see Table II). This is nearly the same as the value obtained by liquid scintillation counting. A comparison of mass spectrometric and liquid scintillation counting specific activities for a similarly labeled cambendazole of lower specific activity and for the C-2 labeled cambendazole is also found in Table II. The second molecular ion cluster in Figure 7 (the first is from unlabeled cambendazole) results from the C-2 labeled drug, and it is evident that this sample is not multiply labeled (note the enhanced intensity of m/e 304 relative to that in the first cluster). In contrast, the ring-labeled (low) drug (third cluster) shows no enhancement of intensity for the m/e peak relative to that of unlabeled drug, but a greatly enhanced intensity at m/e 306. This indicates the presence of two ^{14}C atoms in most of the labeled molecules (but not all, as the ions at m/e 308 and 310 signify species of labeled drug with three and four ^{14}C atoms).

The labeling pattern observed in the spectrum of the high specific activity cambendazole should reflect that locked into the series with the formation of benzene-^{14}C. Scrambling of the carbon-14 of the acetylene during this trimerization is indicated. The observed relative abundances of labeled species (2, 1, 3, 0, 4, 5, 6; number of carbon-14 atoms, in order of decreasing abundance) exactly fits that calculated (by the method described by Rousseau and Mains (1964)) for benzene obtained by the trimerization of acetylene with carbon-14 at an enrichment of 30.5 atom % distributed in a random manner among the species ^{14}C–^{14}C, ^{14}C–^{12}C, and ^{12}C–^{12}C. A different set of relative abundances (2, 0, 4, 1, 3, 6, 5) is expected from the mixture of acetylene richer in ^{14}C–^{14}C with specific activity 55 (mCi/mmol) as charged to the trimerization process. The atomic fractions of carbon-14 used in these calculations were obtained by dividing the specific activity per carbon atom by the specific activity of pure carbon-14 (62.4 mCi/mmol).

The mass spectrometric determination of specific activities of ^{14}C-labeled compounds should not be considered just a curiosity or a tour de force. High specific activity compounds are in demand for a variety of reasons, and with the preparation of very small quantities of ^{14}C-labeled compounds there may be too little sample upon which to obtain an accurate weight for LSC measurement. Further, if there is no uv absorption to allow mass determination, this further complicates the conventional measurement of specific activity. Impure samples also present a challenge, one which may be successfully met by a GLC–mass spectrometric approach.

EXPERIMENTAL SECTION

Analytical TLC was carried out on 5 × 20 cm glass plates coated with silica gel GF_{254} (E. Merck, Darmstadt, Germany). Plates were scanned with a Varian Aerograph/Berthold, Model LB2722 scanner. Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer (Model 3320) using 0.4% Omnifluor in toluene–ethanol (70:30) as scintillator fluid. Final radioactive purity for each compound was determined by establishing a radioactive profile along the plate by scraping sections from the plate followed by liquid scintillation counting. Specific activities were determined as necessary, not on all intermediates. Purity and specific activity or atom percent enrichment of starting materials were taken as given by the supplier and were not redetermined. Mass spectra were obtained with an LKB Model 9000 using the following settings: source temperature, 250 °C; electron energy, 70 eV; accelerating potential, 3.5 kV; trap current, 60 μA.

The high specific activity aniline hydrochloride was supplied as a special custom preparation, along with details of its synthesis from $Ba^{14}CO_3$ by the American Radiochemical Co., Sanford, Fla. Glycine-^{15}N and glycine-*2-*^{13}C were supplied by Merck Sharp & Dohme, Canada, Ltd., Isotope Division. Glycine-*1-*^{14}C was purchased from ICN, Isotope and Nuclear Division.

Thiabendazole-*ar-U-*^{14}C. A stream of anhydrous HCl was passed over a stirred slurry of aniline-^{14}C hydrochloride (819 mg, 6.32 mmol, 9.5 mCi/mmol, 60.0 mCi) and 4-cyanothiazole (750 mg, 6.82 mmol) in 3.5 ml of *o*-dichlorobenzene as the mixture was heated from ambient to 145 °C over 0.5 h, and then kept at 145–150 °C for 4 h. After cooling 7.5 ml of water was added, and the mixture was stirred until all the solid which had formed during the heating period dissolved. Saturated aqueous $NaHCO_3$ was then added to pH 5 (constant for 10 min). The lower, organic layer was removed, and the aqueous solution was extracted with 5 ml more of *o*-dichlorobenzene, and then 2 × 10 ml of ether, each time with pH adjustment. The remaining ether was removed from the aqueous solution by filtering and brief vacuum concentration. An equal volume of MeOH was added, pH was again adjusted to 5.0, and then 8.9 ml of commercial NaOCl (5.25% NaOCl, 6.28 mmol) was added dropwise over a 20-min period with vigorous stirring. After aging at 25 °C for 10 min Na_2CO_3 (740 mg, 7 mmol) was added in a single portion. The mixture was rapidly brought to boiling, and the MeOH was allowed to distill until the vapor temperature reached 90–95 °C. The resulting slurry was cooled to 25 °C, dilute AcOH was added to pH 7.7, and the product was collected after aging at 0 °C. After washing well with water and air drying (caution, thiabendazole sublimes readily under vacuum) the thiabendazole-*ar-U-*^{14}C weighed 1.19 g (93.5% from aniline-^{14}C hydrochloride); radiochemical yield, 53.9 mCi (89.8%).

5-Nitrothiabendazole-*ar-U-*^{14}C. Cold concentrated H_2SO_4 (3.05 g) was added to thiabendazole-^{14}C (1.05 g, 5.24 mmol, 9.1 mCi/mmol, 47.7 mCi) while cooling in an ice bath. The mixture was stirred and warmed to 40–50 °C, whereupon complete solution resulted. The mixture was then chilled to −10 °C for 0.25 h as a solution of concentrated HNO_3 (0.51 ml, 8 mmol) in H_2SO_4 (127 mg) was added dropwise to it, keeping the temperature at −10 °C (approximately 0.5 h required). The mixture was then

allowed to warm to 25 °C and after 0.75 h at that temperature 9.3 ml of hot water was added as the mixture was simultaneously immersed in a bath at 80–85 °C. After 1 h at 80–85 °C the desired 5-NO_2 isomer had crystallized as the sulfate salt, leaving the 4-NO_2 isomer (4–5%) in solution. The product was collected after 1 h at 25 °C, washed well with cold water, and air dried. This solid (1.47 g) was suspended in 50 ml of MeOH–water (1:1) and the slurry was heated to reflux, followed by the addition of aqueous NaOH (2 N, 3.3 ml). MeOH was then slowly distilled until the vapor temperature came to 90–95 °C (3 h). After cooling the pH was adjusted to 7.5 with dilute HOAc. The product, 5-nitrothiabendazole-*ar-U*-^{14}C, collected after aging, weighed 1189 mg (92%). TLC (on SiO_2, benzene–MeOH (3:1)) showed a single spot, radiochemically pure by radioscan.

5-Aminothiabendazole-*ar-U*-^{14}C. A mixture of 1188.7 mg (4.84 mmol) of 5-nitrothiabendazole-^{14}C in 35 ml of MeOH with 168 mg of 10% Pd/C catalyst was shaken and heated at 60–65 °C under 40 psi of H_2 for 4 h. The catalyst was removed by filtration of the hot reaction mixture, handling under an N_2 atmosphere. TLC analysis by radioscan of the solution showed 5-aminothiabendazole of about 95% purity. This material was used for preparation of cambendazole without isolation or further purification.

Isopropyl 2-(4-Thiazolyl)-5-benzimidazolecarbamate-*ar-U*-^{14}C (Cambendazole-*ring*-^{14}C). A MeOH solution of crude 5-aminothiabendazole-^{14}C (from 4.84 mmol of 5-nitrothiabendazole-^{14}C) was treated with 890 mg (0.83 ml, 7.25 mmol) of isopropyl chloroformate, added all at once, followed by an equal volume of water. When the pH had fallen to 2–2.5 dilute aqueous NaOH was added to neutrality. As the pH again fell to 2–2.5 it was adjusted to neutral. When the pH remained stable at 7, TLC of the mixture showed the absence of starting 5-aminothiabendazole. Removal of the MeOH by distillation (760 mm) and readjustment of the pH to 7 with a little HOAc gave 1.33 g, 95.5%, of almost pure cambendazole-^{14}C (specific activity 9.0 mCi/mmol). The radiochemical yield was 39.6 mCi, 73.5% from thiabendazole-*ar-U*-^{14}C. The product may be further purified by conversion to the HCl salt, followed by reconversion to free base.

Isopropyl Chloroformate-^{14}C and Cambendazole-*carbonyl*-^{14}C. 2-PrOH (5 ml) was added directly to 198 mg (2 mmol) of frozen phosgene-^{14}C (5.0 mCi/mmol, 10.0 mCi). The mixture was transferred from the break-seal tube in which first reaction was done to a 250-ml reaction flask with 5 × 1 ml more 2-PrOH. After 1 h at room temperature 432 mg (2 mmol) of 5-aminothiabendazole was added as a solution in warm 2-PrOH (50 ml). After aging for 1 hr crystalline cambendazole hydrochloride separated. TLC showed about 40% unreacted 5-aminothiabendazole (total solids). Re-solution in MeOH–water (1:1) and further acylation with unlabeled isopropyl chloroformate gave cambendazole-*carbonyl*-^{14}C (1.9 mCi/mmol), which was isolated and purified as aforedescribed; radiochemical yield, 61%; chemical yield, 95% (on 5-aminothiabendazole).

Ethyl Glycinate-2-^{13}C Hydrochloride. Glycine-2-^{13}C (2681 mg, 35.7 mmol, 61.0 atom % ^{13}C) was dissolved in 25 ml of anhydrous EtOH saturated with HCl. The mixture was heated to boiling and a gentle flow of HCl gas was passed in sub-surface as 20 ml of solvent was distilled. Ethanol was distilled off, followed by 4 × 25 ml more portions of HCl saturated EtOH which were distilled off at atmospheric pressure. Residual solid was dried under N_2 stream overnight and then slurried in 75 ml of ether and collected. After vacuum drying the product ester hydrochloride weighed 4.77 g (96.2%).

Ethyl *N*-Formylglycinate-2-^{13}C. To a mixture of 34 ml of 98% formic acid and 5.4 ml of Ac_2O was added 4.77 g (34.1 mmol) of ethyl glycinate-2-^{13}C hydrochloride. After 5 min of stirring an additional 54 ml of Ac_2O was added, followed by 1 h more at 25 °C. A solution of 15.5 g of NaOOCH, 29 ml of HOOCH, and 4 ml of Ac_2O was then added to the glycinate solution over a 0.5-h period. The resulting mixture was then carefully heated to 70–75 °C (over 0.75 h), and maintained at this temperature for 0.5 h more, then cooled and aged overnight at room temperature. Solvents were removed (<80 °C, 0.5 mm), leaving an oily solid. This residue was extracted 3 × 150 ml with ether. The ether extract was washed with 200 ml of 1 N NaOH, followed by 2 × 100 ml of water. The ether solution was then dried ($MgSO_4$), and the solvent removed in vacuo, leaving 3.8 g of crude product. This material was distilled at 0.5 mm (160–170 °C (bath)) to yield 3.69 g (82.5%) of pure ethyl *N*-formylglycinate-2-^{13}C (vapor temperature 122–125 °C).

Ethyl Formamidomalonaldehydate-2-^{13}C. Benzene (10 ml) was added to 1.79 g (33.2 mmol) of NaOMe in an N_2 flushed flask. The mixture was stirred and cooled to the freezing (mush) point. Ethyl formate (10 ml) was added with further cooling such that the mixture temperature approximated that of the cooling bath (−10 °C). Ethyl *N*-formylglycinate-2-^{13}C (3.70 g, 28.2 mmol) was then added in three equal portions, maintaining the mixture at −10 °C. Addition of 3 × 2 ml benzene rinse completed the glycinate transfer and gave a stirrable mixture. After 2 h stirring at −10 °C the mixture was stored at 0 °C for 72 h and then diluted with 250 ml of cold ether. The resulting solid was crushed and collected on a filter, washed well with ether, and dried in vacuo. The crude dry salt mixture was added to a mixture of 10 ml of $CHCl_3$, 3.2 ml of concentrated HCl, and 3.2 ml of water at −10 °C. The $CHCl_3$ layer was separated after the mixture was stirred for 0.25 h at −10 °C; the aqueous raffinate was extracted with additional cold $CHCl_3$ (7 × 10 ml). The $CHCl_3$ extracts were dried (cold) over $MgSO_4$, filtered, and concentrated (<25 °C) to yield 3.69 g (82.5%) of ethyl formamidomalonaldehydate-2-^{14}C, a wax-like solid.

Ethyl 4-Thiazole-4-^{13}C-carboxylate. Ethyl formamidomalonaldehydate-2-^{14}C (3.69 g, 23.2 mmol) was combined with 1.16 g (5.2 mmol) of P_2S_5 and dissolved in 22 ml of dry pyridine. The mixture was stirred and heated at 80 °C (bath) for 16 h. A viscous oil separated during this time. Pyridine was removed under moderate vacuum (20 mm, 60 °C). The viscous residue was leached with 5 × 30 ml of ether. The ether extract was dried ($MgSO_4$) and then evaporated to leave an oil which distilled in vacuo to give 2.61 g (71.6%) of ethyl 4-thiazole-4-^{13}C-carboxylate. Upon standing this distillate crystallized, mp 50–53 °C.

4-Thiazole-4-^{13}C-carboxylic Acid. To 2.61 g (16.6 mmol) of ethyl 4-thiazole-4-^{13}C-carboxylate was added 4.8 ml of 4 N aqueous NaOH. Upon warming the mixture gently on a steam bath the solid first melted, then rapidly dissolved accompanied by a vigorous exotherm. The mixture was heated 0.25 h longer, cooled to 25 °C, aged for 0.75 h, and then with cooling treated with HCl to pH 1. The resulting mixture was evaporated and finally dried over P_2O_5. The crude product, with inorganic salts, was used as such for preparation of acid chloride.

4-Thiazole-4-^{13}C-carboxylyl Chloride. Crude, dry 4-thiazole-4-^{13}C-carboxylic acid (from 2.6 g of ester) was treated with 20 ml of $SOCl_2$, warmed slowly to reflux, and refluxed for 3 h more. Excess $SOCl_2$ was distilled, re-

moving the residual reagent by distilling 3 × 20 ml of benzene. The residue was suspended in 50 ml of hot benzene and filtered (removes inorganic salts). Benzene was removed, and the residue was extracted with 50 ml of boiling cyclohexane (leaves tar). Removal of the cyclohexane, and careful vacuum drying (product may sublime), gave 2.12 g (86.2% from the ester) of 4-thiazole-4-^{13}C-carboxylyl chloride, mp 83–88 °C (lit. mp 87–89 °C).

Isopropyl 3-Nitro-4-(4-thiazole-4-^{13}C-carboxamido)carbanilate. A solution of 1.18 g (8 mmol) of 4-thiazole-4-^{13}C-carboxylyl chloride and 1.91 g (8 mmol) of isopropyl 4-amino-3-nitrocarbanilate in 13 ml of dry pyridine was warmed to 100 °C for 3 h and then poured into 1 l. of EtOAc and heated to boiling, dissolving the precipitated yellow solid. The solution was extracted with 1 N HCl, with aqueous $NaHCO_3$, and then water, and finally dried ($MgSO_4$), all while still warm. After concentration in vacuo to a thick slurry and cooling (0–5 °C) the product was collected, washed with cold EtOAc, and dried to obtain 2.44 g (87.5%) of isopropyl 3-nitro-4-(4-thiazole-^{13}C-carboxamido)carbanilate.

Isopropyl 3-Amino-4-(4-thiazole-4-^{13}C-carboxamido)carbanilate. Two 1.22-g portions of isopropyl 3-nitro-4-(4-thiazole-4-^{13}C-carboxamido)carbanilate, each with 250 mg of 5% Pd/C in 300 ml of 2-PrOH, were reduced under H_2 at 40 psi at 65–70 °C for 16 h. The product amine precipitated as formed. The two runs were combined, concentrated to 25 ml total volume, and used directly (with catalyst remaining in) in the following cyclization procedure.

Isopropyl 2-(4-Thiazolyl-4-^{13}C)-5-benzimidazolecarbamate (Cambendazole-*thiazole-4*-^{13}C). The previously described slurry of isopropyl 3-amino-4-(4-thiazole-4-^{13}C-carboxamido)carbanilate (from 2.44 g of nitro compound) was treated with 10 ml of concentrated HCl whereupon the mixture set up into an unstirrable paste. This was mobilized by the addition of 75 ml of 2-PrOH and then was refluxed for 5 h. The mixture was cooled, 10 ml of concentrated NH_4OH was added followed by 100 ml of EtOAc, and the mixture was again heated to boiling. After 0.5 h the hot mixture was filtered through Celite (removing the Pd/C catalyst), washing the cake with 4 × 200 ml of hot EtOAc. After removal of a small aqueous layer the solution was dried ($MgSO_4$) and then concentrated to dryness, giving 2.21 g of crude cambendazole-*thiazole-4*-^{13}C.

The crude product was dissolved in 12 ml of hot HOAc. On cooling and seeding the HOAc solvate of CBZ crystallized. This was collected, washed with cold HOAc and then with ether, and dried (1.88 g). The solvate was dissolved in 100 ml of MeOH–water (1:1), and the resulting solution made just alkaline with 212 ml of 2.5 N NaOH. Solvent was removed by boiling (atmospheric) until a vapor temperature of 90 °C was obtained. The resulting slurry was cooled to 25 °C, pH was adjusted to 7.0, and the product was collected and washed with water. So obtained was 1.38 g (65.4% from the nitro intermediate) of cambendazole-*thiazole-4*-^{13}C (57.0 atom % ^{13}C).

In a manner exactly the same as that described for transformation of glycine-2-^{13}C to cambendazole-*thiazole-4*-^{13}C samples of glycine-*1*-^{14}C and glycine-^{15}N (32 atom % ^{15}N) were used to prepare cambendazole-2-^{14}C (2.8 mCi/mmol) and cambendazole-*thiazole*-^{15}N (33 atom % ^{15}N), respectively. The following labeled intermediates were involved: ethyl glycinate-*1*-^{14}C hydrochloride (2.8 mCi/mmol); ethyl *N*-formylglycinate-*1*-^{14}C (2.8 mCi/mmol); ethyl formamidomalonaldehydate-*1*-^{14}C (2.8 mCi/mmol); ethyl 4-thiazolecarboxylate-^{14}C (2.8 mCi/mmol); 4-thiazolecarboxylic-^{14}C acid (2.8 mCi/mmol); 4-thiazolecarboxylyl-^{14}C chloride (2.8 mCi/mmol); isopropyl 3-nitro-4-(4-thiazolecarboxamido-^{14}C)carbanilate (3.2 mCi/mmol); isopropyl 3-amino-4-(4-thiazolecarboxamido-^{14}C)carbanilate (3.2 mCi/mmol); ethyl ^{15}N-glycinate hydrochloride (33 atom % ^{15}N); ethyl ^{15}N-formylglycinate (33 atom % ^{15}N); ethyl formamido-^{15}N-malonaldehydate (33 atom % ^{15}N); ethyl 4-thiazole-^{15}N-carboxylate (33 atom % ^{15}N); 4-thiazole-^{15}N-carboxylic acid (33 atom % ^{15}N); 4-thiazole-^{15}N-carboxylyl chloride (33 atom % ^{15}N); isopropyl 3-nitro-4-(4-thiazole-^{15}N-carboxamido)carbanilate (33 atom % ^{15}N); isopropyl 3-amino-4-(4-thiazole-^{15}N-carboxamido)carbanilate (33 atom % ^{15}N).

NOTE ADDED IN PROOF

The mass spectrometric determination of ^{14}C specific activity in labeled cholesterol has recently been reported (Burstein et al., 1975).

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