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Synthesis of Radiolabeled Ethyl *O*-Benzoyl-3-chloro-2,6-dimethoxybenzohydroximates: Oxime-^{14}C, Carbonyl-^{14}C, and 2-Methoxy-^{14}C

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Ethyl *O*-benzoyl-3-chloro-2,6-dimethoxybenzohydroximate, benzomate acaricide, was prepared with specific ^{14}C-labeling at the oxime, carbonyl, and 2-methoxy positions. Benzomate-*oxime*-^{14}C (0.396 mCi/mmol) was prepared with the overall radiochemical yield of 4.2% from acetic anhydride-*1*-^{14}C and 4-methoxy-7-hydroxycoumarin through nine-step reactions. Benzomate-*carbonyl*-^{14}C (1.13 mCi/mmol) was prepared with the yield of 32.2% by a one-step reaction, which gave preferentially an *N*-benzoyl isomer with rather high yield (49.8%). Benzomate-*2-methoxy*-^{14}C (0.117 mCi/mmol) was prepared in four-step reactions, starting from methylation by a substituted salicylate with diazomethane-^{14}C.

Benzomate (ethyl *O*-benzoyl-3-chloro-2,6-dimethoxybenzohydroximate) is a new acaricide developed by Nippon Soda Co., Ltd., and effective especially against *Panonychus citri* (Noguchi *et al.*, 1971). The study of the metabolic fate of benzomate is facilitated by the use of radiolabeled materials in different parts of the molecule. It was recently found by using these labeled compounds that the debenzoylation of benzomate occurred moderately on citrus fruit (Soeda *et al.*, 1971). This report deals with the synthesis of benzomate labeled with ^{14}C at different positions.

By either acid or alkaline hydrolysis, benzomate is easily debenzoylated to give ethyl 3-chloro-2,6-dimethoxybenzohydroximate. On heating in 47% hydroiodic acid, debenzoyl benzomate is demethylated exclusively at the 2-methoxy grouping and the resulting ethyl 3-chloro-2-hydroxy-6-methoxybenzohydroximate was resistant to further demethylation (Tohyama *et al.*, 1970).

Of the several groupings retained in the major skeleton of the molecule under these chemical reactions, the oxime carbon was selected for labeling, since it was supposed to survive with the benzene ring in the metabolic process. In addition, two chemically labile groupings, carbonyl and 2-methoxy, were also selected for labeling. With those differently labeled compounds available, a metabolite will be more easily identified by the presence or absence of radioactivity, depending on the kind of the starting labeled compounds. Therefore the three differently labeled benzomates were synthesized.

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The synthetic schemes are shown in Figure 1. According to the known procedures (Russell and Frye, 1955), nonlabeled resorcinol was converted to 2,6-dihydroxyacetophenone-^{14}C (**4**) *via* 4-methyl-7-acetoxycoumarin-^{14}C (**3**), the radioactive carbon of which had been introduced by acetic anhydride-*1*-^{14}C with some modification. Acetylation by the known procedure of 4-methyl-7-hydroxycoumarin (**1**) with acetic anhydride without any solvent gave 95% yield of the product in a preliminary cold run. Acetic anhydride-^{14}C, however, was only available in benzene, which was a poor solvent for the compound to be acetylated. Therefore pyridine had to be added for the reaction to take place.

In the preliminary study, methylation of 2,6-dihydroxyacetophenone-^{14}C (**4**) with dimethyl sulfate in 2 *N* sodium hydroxide gave a mixture of mono- and dimethyl derivatives. Therefore the methylation was actually accomplished in two steps. Compound **4** was treated with excess diazomethane to give the monomethyl derivative, which was further methylated with dimethyl sulfate in 4 *N* alkaline solution. In view of this result, however, it is likely that 2,6-hydroxydiacetophenone can be fully methylated in one step at both phenolic hydroxyl groupings with dimethyl sulfate, if 4 *N* sodium hydroxide is used.

The monomethyl derivative, 6-hydroxy-2-methoxyacetophenone, was soluble in benzene but sparingly soluble in alkaline water. When the silica gel tlc had been developed with benzene, the R_f value (0.37) of this compound was greater than that (R_f 0.21) of the dimethyl derivative **5**. So, this compound might have an intramolecular hydrogen bonding (>C=O · · · H–O–).

The same reagent, sodium hypochlorite, was used for the oxidation and the subsequent chlorination of 2,6-dimethoxyacetophenone (**5**), so the latter reaction could be performed either with or without isolation of 2,6-dimethoxybenzoic acid (**6**). In either case the chemical yield was found to be around 60%. 3-Chloro-2,6-dimethoxybenzoic acid (**7**) thus obtained

was reacted with thionyl chloride and the resulting acid chloride was converted to debenzoyl benzomate (**8**) by the reaction with ethoxyamine.

Benzoylation of *N*-ethoxy-3-chloro-2,6-dimethoxybenzoamide (**8**, **10**, **15**) in the presence of triethylamine gave preferentially (80–90% yield) *O*-benzoylated product, while in the absence of the base *N*-benzoylation became predominant. Thus, benzomate-*oxime*-^{14}C, having a radiochemical purity of 99%, was prepared with the overall chemical yield of 4.2% and radiochemical yield of 3.3% from acetic anhydride-*1*-^{14}C in nine-step reactions.

Benzomate-*carbonyl*-^{14}C was prepared in radiochemical purity of 99% according to the procedure already mentioned in the synthesis of benzomate-*oxime*-^{14}C with an overall chemical yield of 35.5% and radiochemical yield of 32.3% (Figure 1, B).

Figure 1, C shows the synthetic scheme for 2-methoxy-^{14}C-benzomate. Diazomethane generated from *N*-methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide was introduced into ethereal 3-chloro-2-hydroxy-6-methoxybenzoic acid and the resulting ester was hydrolyzed. 2-Methoxy-^{14}C-3-chloro-2,6-dimethoxybenzoic acid thus obtained was subjected to the series of reactions already described to give benzomate-2-methoxy-^{14}C, having a radiochemical purity of 99%, with an overall chemical yield of 24.8% and a radiochemical yield of 20.3%.

Table I summarizes the data of the chemical and radiochemical yield, the radiochemical purity, and the specific activity of each compound, together with the yields of nonlabeling synthesis. The purer the starting materials, the better the results obtained were, as usually is the case in organic synthesis. Each specific activity of benzomate-^{14}C obtained was in good agreement with the calculated one except for compound **14**, where an unknown chemical impurity might be present as judged by the difference of chemical yield from the radiochemical one.

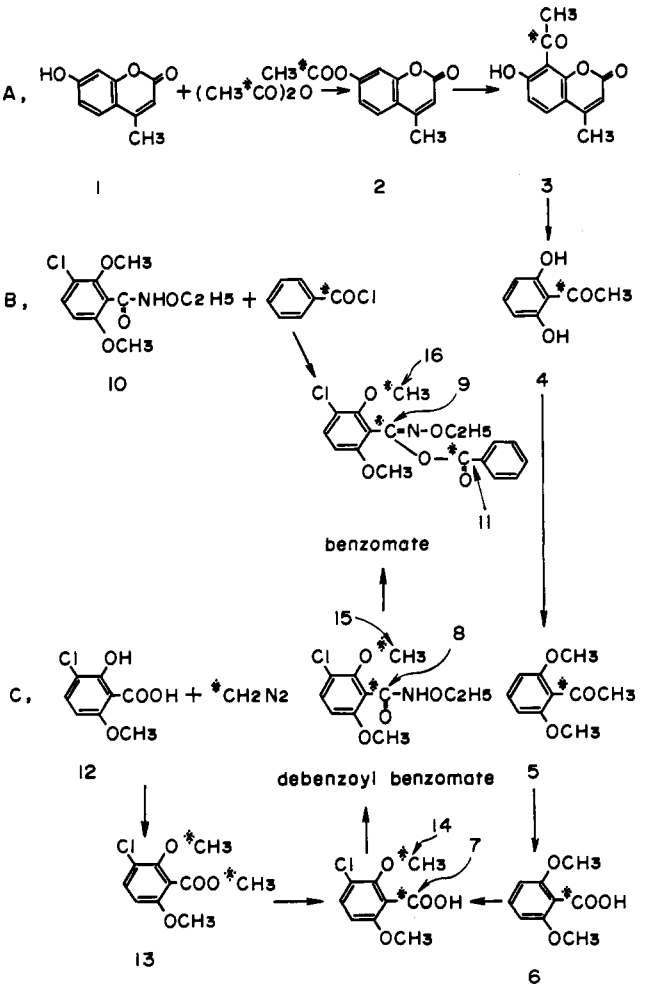

Figure 1. Scheme for the synthesis of *oxime*-^{14}C (A), *carbonyl*-^{14}C (B), and 2-*methoxy*-^{14}C (C)-labeled benzomates

EXPERIMENTAL

Starting Radiochemicals. Three kinds of starting radiolabeled compounds were purchased and used without further purification. Acetic anhydride-*1*-^{14}C, 45.9 mCi/mmol, and benzoic acid, 10.2 mCi/mmol, were obtained from Daiichi Pure Chemicals Co., Ltd., Tokyo, and both of the compounds had the radiochemical purity of 99%. *N*-(Methyl-^{14}C)-*N*-nitroso-*p*-toluenesulfonamide, 1.64 mCi/mmol, was obtained from The Radiochemical Centre, Amersham, England, and had the radiochemical purity of 99%.

Authentic Standards and Starting Nonlabeled Compounds. Nonlabeled authentic standards of benzomate and its synthetic intermediates, **5**, **6**, **7**, **8**, **9**, and **13** in Figure 1, were supplied by Fine Chemical Research Laboratories, Nippon Soda Co., Ltd., Odawara: mp of **5**, 66–68°; **6**, 185–187°; **7**, 133–135°; **8**, 125–126°; **9**, 69–71°; **13**, 87–89°. The infrared and nmr spectra of benzomate are shown in Figures 2 and 3, respectively. The data of the elemental analysis of benzomate were as follows. Calculated for $C_{18}H_{18}ClNO_5$: C, 59.42; H, 4.95; N, 3.85; Cl, 9.77. Found: C, 59.50; H, 5.10; N, 4.08; Cl, 9.37. Compound **12** synthesized from potassium 2-chloro-6-methoxyphenolate and carbon dioxide was kindly supplied by Kohhei Hashimoto, Nisso Takaoka Works, Nippon Soda Co., Ltd., Takaoka; mp 133.3–137°.

Identification of Labeled Compounds. Synthetic labeled compounds was identified by thin-layer chromatography (tlc) with authentic standards. Tlc was performed on the poly-

Table I. Data Sheet of Chemical and Radiochemical Syntheses of Benzomate

Reaction equation		Chemical		Radiochemical		Specific activity		Radiochemical purity, %
		Amount, mg	Yield, %	Activity, mCi	Yield, %	Calcd (mCi/mmol)	Obsd (mCi/mmol)	
A	(1)–(2)	738.2	56.3 (70.0)[a]	2.27	56.0	0.666	0.654	99
	(2)–(4)	577.0	75.5 (76.0)	1.63	73.5	0.464	0.429	87.5
	(4)–(5)	254.0	37.2 (40.0)	0.556	34.1	0.464	0.394	98
	(5)–(7)	225.3	49.4 (58.0)	0.223	40.1	0.292	0.291	95.2
	(7)–(8)	225.0	83.3 (84.7)	0.189	84.6	0.292	0.283	99
	(8)–(9)	159.0	64.8 (92.0)	0.130	69.0	0.292	0.296	99
B	(10)–(11)	51.4	35.5 (44.2)	0.161	32.2	1.110	1.14	99
C	(12)–(14)	93.3	46.1 (26.1)	0.036	28.6	0.133	0.083	99
	(14)–(16)	82.0	54.3 (77.7)	0.027	73.3	0.133	0.118	99

[a]Figures in parentheses mean the chemical yield in nonlabeled syntheses using pure materials in each step.

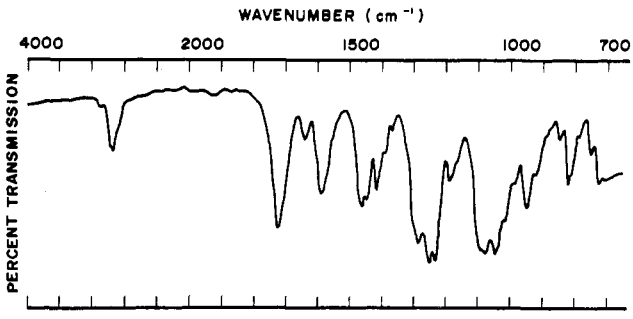

Figure 2. Infrared spectrum of benzomate. Measured in KBr (1.25%)

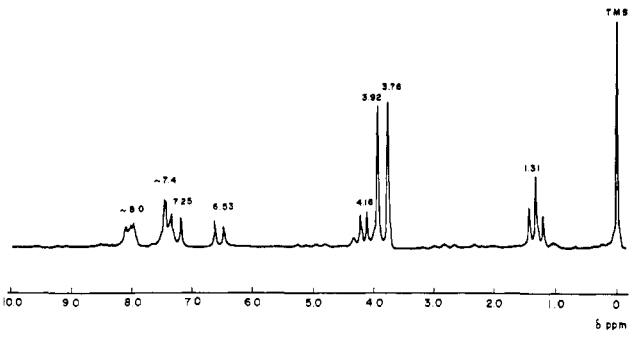

Figure 3. Nuclear magnetic resonance spectrum of benzomate. Measured in CCl_4 at 60 MHz. Figures mean ppm from TMS

ethylene film coated with a silica gel layer (Eastman chromagram sheet 6060). The solvent systems used are mentioned in each synthetic step. The radiochemical purity of each labeled compound was determined by tlc–autoradiogram and by counting the radioactivity of tlc spots by a liquid scintillation counter (Packard Tri-Carb 3320) after extraction.

Benzomate-*oxime*-^{14}C (9). 4-METHYL-7-ACETOXYCOUMARIN-^{14}C (2). Acetic anhydride-*1*-^{14}C (8 mCi, 17.8 mg in 4 ml of benzene) was transferred from an ampoule to a 10-ml reaction flask with 1 ml of benzene and nonlabeled acetic anhydride (594.8 mg, total 6 mmol). Dry coumarin (1) (1300 mg, 7.5 mmol, mp 184–185°) and 2.4 g of dry pyridine were added to the benzene solution containing acetic anhydride-*1*-^{14}C. The mixture was refluxed for 1 hr at 140 ± 2° on a oil bath. After the reaction mixture had been condensed, each 5 ml of water and an aqueous solution of 10% sodium hydroxide was poured into the residue under stirring. The precipitates were filtered and recrystallized from 4 ml of 95% ethanol and dried in a P_2O_5 desiccator. Yield was 738.2 mg (56.3%). The radiochemical purity of 99% was obtained when the tlc plate was developed with ethyl acetate (R_f 0.73).

2,6-DIHYDROXYACETOPHENONE-^{14}C (4). The mixture of acetoxycoumarin-^{14}C (2) (737 mg) diluted with nonlabeled compound (302 mg, total 5.05 mmol) and anhydrous aluminum chloride (2301 mg, 17.2 mmol) was heated at 170° for about 2 hr to give compound 3. The crude crystals of compound 3 were mixed with 15 ml of 5% aqueous sodium hydroxide and the mixture was heated at 95 ± 2° for 5 hr under nitrogen atmosphere. The solution was then cooled and acidified by the addition of 6.5 ml of 10% aqueous hydrochloric acid. The crude 2,6-dihydroxyacetophenone-^{14}C (4) which separated on acidification was collected on a filter paper, washed with cold water, and dried in a P_2O_5 desiccator. Yield was 577 mg (77.5%). The radiochemical purity of 87.5% was obtained when the tlc plate was developed with acetone–ethyl acetate (3:7) (R_f 0.68).

2,6-DIMETHOXYACETOPHENONE-^{14}C (5). Crude dihydroxyacetophenone-^{14}C (4) (577 mg, 3.8 mmol, purity 87.5%) was dissolved in 33 ml of ethyl ether containing diazomethane (660 mg, 15.7 mmol) and was then allowed to stand for 10 hr at room temperature. The evaporation of ether gave the residue which was found to be a mixture of three compounds, dihydroxyacetophenone and its mono- and dimethyl ether. So, the residue was again methylated with dimethyl sulfate (1.51 g, 12.0 mmol) and 7.5 ml of 2 *N* aqueous sodium hydroxide (total 15 mmol) at 95° for 1 hr. The mixture was extracted with benzene. The benzene was evaporated and the residue was chromatographed by a silica gel–*n*-hexane–benzene system (20 g, 2 cm in diameter) to isolate the monomethyl derivative (87 mg, fractions 25–48, each 10 ml) and the dimethyl derivative (204 mg, fractions 52–55). The monomethyl derivative was again methylated with dimethyl sulfate (317 mg) and 0.8 ml of 4 *N* aqueous sodium hydroxide to obtain second crops of dimethyl derivative (65 mg). The combined crystals of dimethoxyacetophenone were purified through the same column chromatography. Yield was 254 mg (34.1%). The radiochemical purity of 98% was obtained when the tlc plate was developed with benzene (R_f 0.21).

3-CHLORO-2,6-DIMETHOXYBENZOIC ACID-^{14}C (7). The mixture of 2,6-dimethoxyacetophenone-^{14}C (5) (254 mg, 1.41 mmol), 4.6 g of sodium hypochlorite solution containing 5.9% of chlorine and 10% of sodium hydroxide in w/w, and 0.88 g of 28% aqueous sodium hydroxide was heated at 85° for 3 hr. After the mixture had been cooled, it was acidified with concentrated hydrochloric acid, and was left for 1 hr. The precipitate which appeared was collected on a filter paper and washed with water followed by benzene, and then air dried. Yield was 164 mg.

The mixture of crude 2,6-dimethoxybenzoic acid-^{14}C (164 mg) diluted with nonlabeled compound (80.5 mg, total 1.34 mmol) and 4.06 g of sodium hypochlorite solution containing 3.4% of chlorine and 4% of sodium hydroxide in w/w was kept at 10–15° for 2 hr. After the filtration of the mixture and acidification of the filtrate with concentrated hydrochloric acid, the solution was kept at 10–15° for 1 hr and extracted by ethyl ether. The ethereal solution was evaporated to give 3-chloro-2,6-dimethoxybenzoic acid-^{14}C (7), 225.3 mg (49.4%). The radiochemical purity of 95.2% was obtained when the tlc plate was developed with ethyl acetate–methanol–acetic acid (50:50:0.5) (unsaturated R_f 0.50).

ETHYL 3-CHLORO-2,6-DIMETHOXYBENZOHYDROXIMATE-^{14}C (8). 3-Chloro-2,6-dimethoxybenzoic acid-^{14}C (7) (225.3 mg, 1.040 mmol) was heated with thionyl chloride (600 mg, 5 mmol) at 80° for 30 min. After evaporating the excess thionyl chloride *in vacuo*, the residue was well mixed with 1.23 g of toluene and a mixture of 0.5 ml of 26.4% aqueous ethoxyamine (2 mmol) and 525 mg of 8% aqueous sodium bicarbonate. The mixture was left overnight in a refrigerator and then at 15° for 2 hr. After adding an additional 1 ml of 8% aqueous sodium bicarbonate, the mixture was extracted with chloroform. The chloroform was evaporated and the residue was recrystallized from acetone–*n*-hexane (0.5:0.55 ml) to give leaflet crystals, 225 mg (83.3%). The radiochemical purity of 99% was obtained when tlc plate was developed with acetone–methanol (9:1) (R_f 0.78).

BENZOMATE-*oxime*-^{14}C (9). Ethyl 3-chloro-2,6-dimethoxybenzohydroximate-^{14}C (8) (175 mg, 0.675 mmol) was dissolved in 1.5 g of chloroform and then mixed with 130 mg of 28% aqueous sodium hydroxide and 10 mg of triethylamine. The mixture was cooled at −5°, and benzoyl chloride (140 mg, 1 mmol) was added in bulk, and then kept at −5° for 5 hr. Af-

ter separating off the water layer, the chloroform layer was washed with 30% aqueous hydrochloric acid followed by water, dried over anhydrous sodium hydroxide, and then condensed to dryness to give crystals of benzomate and its isomer, the *N*-benzoyl form. The benzomate was purified by silica gel column chromatography (10 g, 12 mm in diameter). The eluting solvent was a mixture of *n*-hexane and acetone (96:4, v/v); 5-ml fractions were collected. Benzomate was eluted in fractions 6–17 (159 mg) and *N*-benzoyl isomer in fractions 24–28 (4.3 mg). Yield was 159 mg (64.8%). The radiochemical purity of 99% was determined on tlc by the next two solvent systems; *n*-hexane–benzene–acetic acid (80:20:2) (unsaturated R_f O isomer 0.20, N isomer 0.12), benzene–ethyl acetate (80:20) (R_f O isomer 0.52, N isomer 0.63).

Benzomate-*carbonyl*-^{14}C (11). Benzoic acid-^{14}C (0.5 mCi) diluted with nonlabeled compound (50 mg, 0.42 mmol) was heated with thionyl chloride (0.70 ml, 9 mmol) at 80° for 30 min. Excess thionyl chloride was evaporated under reduced pressure. The residue was dissolved in 1 ml of chloroform. To this solution, ethyl 3-chloro-2,6-dimethoxybenzohydroximate (103 mg, 0.4 mmol) and 0.22 ml of 25% aqueous sodium hydroxide were added. After mixing well, the mixture was kept in a freezer (−13°) for 24 hr. The chloroform layer was separated and washed with 30% aqueous hydrochloric acid followed by water, dried over anhydrous sodium hydroxide, and then condensed to dryness to give the crystals of benzomate and *N*-benzoyl isomer. These compounds were separated by silica gel column chromatography (packed 10 g, 12 mm in diameter). The solvent for development was a mixture of *n*-hexane and acetone (99:1, v/v); 5–10-ml fractions were collected. Benzomate was eluted in fractions 11–17, and the isomer in fractions 18–27, as checked by tlc. After evaporating the solvent, benzomate was obtained in the yield of 51.4 mg (35.5%) with the radiochemical purity of 99% and the isomer in the yield of 83.0 mg (57.3%). The tlc plates were developed with the same solvents in the case of oxime-^{14}C. Both compounds showed the same specific radioactivity (1.12 mCi/mmol).

Benzomate-2-*methoxy*-^{14}C (16). The solution consisting of 600 mg of sodium hydroxide, 1 ml of water, and 2.5 ml of ethanol was added dropwise to 5 ml of ethanol solution containing *N*-methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide (0.5 mCi, 65 mg) diluted with nonlabeled compound (335 mg, total 2 mmol) at 65°. Liberated diazomethane-^{14}C was introduced into 2 ml of the ethereal solution of 3-chloro-6-methoxysalicylic acid (**12**) (101.5 mg, 0.50 mmol) under ice cooling. After the solution had been kept overnight in an ice–water bath, the ether was evaporated and the residue was mixed with 10 ml of Claisen alkaline solution consisting of 3.5 g of potassium hydroxide in water–methanol (1:3), and then boiled for 2 hr. After acidification with hydrochloric acid, the mixture was extracted with ethyl acetate (5 ml, three times). The extract was dried over anhydrous sodium sulfate and condensed to give 3-chloro-2,6-dimethoxybenzoic acid-^{14}C (**14**), 93.3 mg (43% for sulfonamide). The radiochemical purity of 99% was obtained when the tlc plate was developed with ethyl acetate–methanol–acetic acid (90:10:2) (R_f 0.68).

Crude 3-chloro-2,6-dimethoxybenzoic acid-^{14}C (**14**) (91 mg, 0.42 mmol) was treated with excess thionyl chloride (500 mg, 4.6 mmol) to give the corresponding acid chloride. As described in the synthesis of oxime-^{14}C, the acid chloride was converted to the corresponding ethoxyamido (**15**) (87 mg, 81.7%) by treatment with 1 ml of toluene, 0.4 ml of 8% aqueous sodium bicarbonate, and 0.2 ml of 26.4% aqueous ethoxyamine (0.8 mmol). Then the resulting ethoxyamide (77 mg) was dissolved in 750 mg of chloroform and treated with 66 mg of 28% aqueous sodium hydroxide and 5 ml of triethylamine followed by benzoyl chloride (140 mg) to give benzomate-^{14}C (**16**), which was purified by column chromatography as mentioned in the synthesis of benzomate-*oxime*-^{14}C. Yield was 69.8 mg (66.5%). The radiochemical purity of 99% was obtained when the tlc plates were developed with the same solvents in the case of oxime-^{14}C.

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