SYNTHESES OF RADIOACTIVE AND STABLE ISOTOPE-LABELLED 1-ETHYL-6,7-METHYLENEDIOXY-4(1H)-OXOCINNOLINE-3-CARBOXYLIC ACIDS (CINOXACIN)

T. Nagasaki, Y. Katsuyama, and H. Minato*

Shionogi Research Laboratory, Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

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SUMMARY

1-Ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carboxylic acid (cinoxacin) (I), an effective antimicrobial agent has been labelled with carbon-14. The carbon-14 was incorporated into the C-4 position of the molecule to give XIV in 10.0% overall radiochemical yield based on barium carbonate-^{14}C. The ^{13}C-, ^{15}N-, and d-labelled compounds (XV, XVII, and XVIII) have also been synthesized for absorption and metabolism studies.

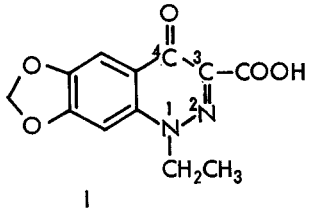
Key Words: 1-Ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carboxylic acid, Cinoxacin, Carbon-13 and -14, Nitrogen-15, Deuterium, ^{13}C FT NMR spectrum, Mass spectrum.

Heretofore, a number of compounds have been used for prevention and treatment of microbial infections arising from Gram-negative bacteria and Mycoplasma organisms. Among such compounds are nalidixic acid and the 1,4-dihydro-4-oxo-3-quinoline-carboxylic acid derivatives.[1,2] However, none of them are effective against all strains of Gram-negative bacteria or Mycoplasma or a combination of the two.

The Lilly Research Laboratories[3] have synthesized cinoxanin (1-ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carboxylic acid; I) as a more effective agent against a broad spectrum of microbial organisms.[4]

In order to conduct absorption and metabolism studies with I, the isotope-labelled drug was required. Although the radioactive compound is used quite often in such studies, it is not suitable for the structural elucidation of metabolites. On the other hand, the stable isotope-labelled compound is also used in biotransformation studies, based on safety in administration to human subjects and utility in identification and quantitative estimation of the metabolites by mass spectrometric techniques. Therefore, we wynthesized not only ^{14}C-labelled cinoxacin* but the stable isotope-labelled one also. Syntheses of all labelled compounds were achieved by the Lilly route[3] indicated in the Scheme.

The carbon-14 label may be incorporated into the C-3 or C-4 position of the cinnoline ring, the ethyl group at N-1, or the carboxyl group at C-3.

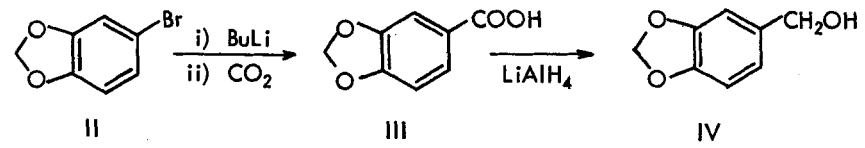


I

However, based on stability relative to metabolic loss and facility of obtaining the ^{14}C-labelled starting material, we labelled the carbon at the 4-position of the cinnoline ring with carbon-14. The ^{14}C-carboxylation of 3,4-methylenedioxybromobenzene (II) was performed from barium carbonate-^{14}C (50 mCi) using butyl lithium to give 3,4-methylenedioxybenzoic acid-1-^{14}C (III) in 88.4% radiochemical yield. 3',4'-Methylenediocyacetophenone-1-^{14}C (VII) was obtained from the acid (III) via the benzyl alcohol (IV), the aldehyde (V), and the alcohol (VI) in 65.5% overall radiochemical yield. Treatment of the acetophenone (VII) with nitric acid (d = 1.42) afforded 2'-nitro-4',5'-methylenedioxyacetophenone-1-^{14}C (VIII) as colourless prisms, m.p. 123-124°, 20.3 mCi in 70% radiochemical yield.

The nitro compound (VIII) was hydrogenated with platinium dioxide as a catalyst in

ethanol to give the amino derivative (IX). A solution of the amino compound (IX) in hydrochloric acid was treated with sodium nitrite, affording 4-hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (X), from which the 3-cyano-derivative (XII), 13.6 mCi, was derived via the 3-bromo-derivative (XI). These products were all obtained in successful radiochemical yield.



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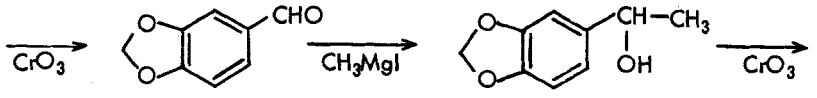
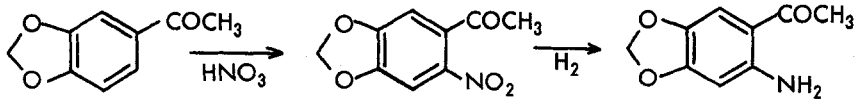
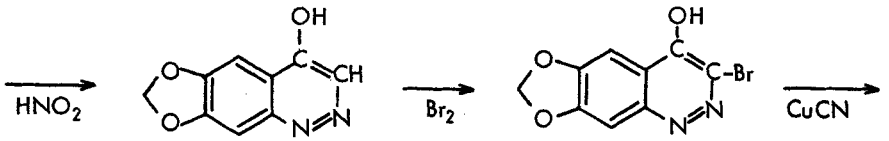


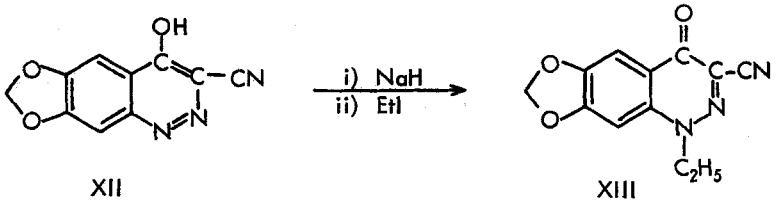
V VI



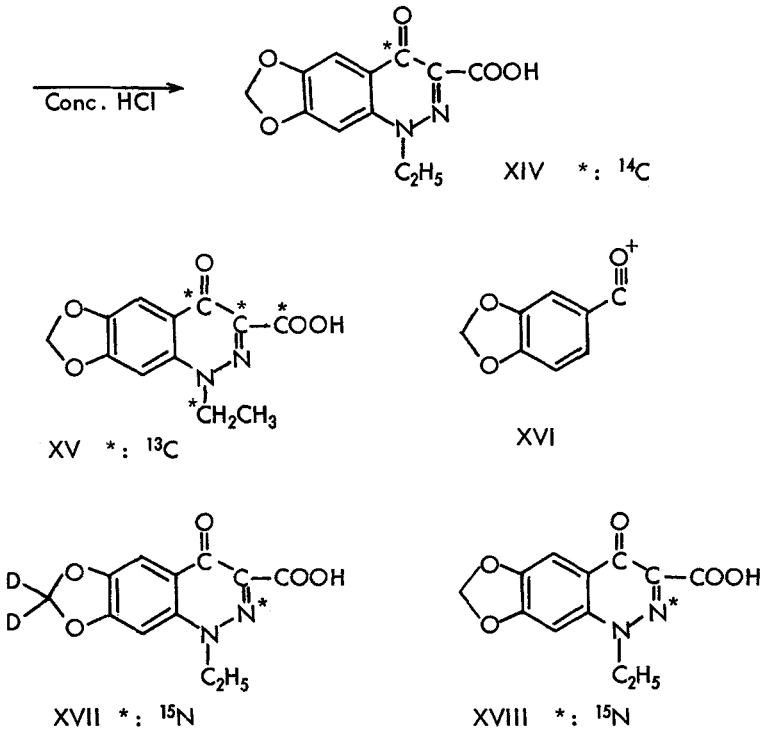
VII VIII IX



X XI



XII XIII



Scheme

When the sodium salt of the 3-cyano-derivative (XII) was heated with ethyl iodide in dimethylformamide, it gave 1-ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carbonitrile-4-^{14}C (XIII) as a yellow brown powder in 89% radiochemical yield. Cinoxacin-^{14}C (XIV) was obtained by hydrolysis of XIII with hydrochloric acid and acetic acid as pale yellow crystals, m.p. 261-262° (dec.), 5 mCi (specific activity: 3.42 mCi/mM) in 41.3% radiochemical yield. Overall radiochemical yield based on barium carbonate-^{14}C was 10.0%.

In the metabolism study with the stable isotope-labelled compound, the structures of some metabolites can be found by mass spectra. However, ^{13}C NMR spectroscopy may be more powerful for structural elucidation of the metabolite. As the product of the biotransformation is obtained only in small amounts, ^{13}C NMR spectral measurement is restricted by the amount of sample available. On the assumption that 100-200 mcg of the sample serves this purpose, the reagents enriched with about 90 atom% C-13 (M.S.D. Co.) were used to label all carbons at the 3- and 4-positions of the cinnoline ring, the carboxyl group at C-3,

and the methylene of the ethyl group at N-1.

^{13}C-carboxylation of 3,4-methylenedioxybromobenzene (II) was performed from barium carbonate-^{13}C (90.7 atom %) in the same manner as in the case of the ^{14}C-carboxylation to give 3,4-methylenedioxybenzoic acid-1-^{13}C (III) in 72% yield. The ^{1}H-noise-decoupled ^{13}C FT NMR spectrum of this acid verified the location of the ^{13}C-label by the fact that only one singlet due to the carboxyl carbon was observed at δ 166.3. The acid (III) was reduced with lithium aluminium hydride to the benzyl alcohol-^{13}C (IV) in 96.5% crude yield. The ^{13}C NMR spectrum showed a signal due to the benzylic carbon as a singlet at δ 65.2. The alcohol (IV) was oxidized with chromium trioxide-pyridine to give 3,4-methylenedioxybenzaldehyde-1-^{13}C (V) in 90% yield, which displayed the aldehyde carbon signal as a singlet at δ 189.9 in the ^{13}C NMR spectrum. 1'-Hydroxyethyl-3,4-methylenedioxybenzene-1',2'-^{13}C (VI) was obtained by Grignard reaction of the aldehyde (V) with 1.35 equivalent moles of methyl iodide-^{13}C (90 atom %) as a colourless oil in quantitative yield. The ^{13}C NMR spectrum showed a doublet at δ 70.3 (J = 39 Hz) due to the benzylic carbon and a doublet at δ 25.2 (J = 39 Hz) due to the methyl carbon. Oxidation of the alcohol (VI) with chromium trioxide-pyridine afforded 3',4'-methylenedioxyacetophenone-1,2-^{13}C (VII) as colourless prisms, m.p. 86-87°, in 94% yield. The ^{13}C NMR spectrum confirmed the structure of the product, exhibiting a doublet at δ 196.2 (J = 44 Hz) and another at δ 26.4 (J = 44 Hz).

In the same manner as for the ^{14}C derivative, 2'-nitro-(VIII) and 2'-amino-4',5'-methylenedioxyacetophenone-1,2-^{13}C (IX) were synthesized in 71% and 90% yield, respectively. The ^{13}C NMR spectra of VIII and IX showed a doublet at δ 198.7 (J = 44 Hz) and δ 197.7 (J = 43 Hz) due to the carbonyl carbon, and a doublet at δ 30.1 (J = 44 Hz) and δ 27.8 (J = 43 Hz) due to the methyl carbon, respectively. 4-Hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (X) and 3-bromo-4-hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (XI) were synthesized as shown in the Scheme. The carbon at the 4-position was observed as a doublet at δ 168.2 (J = 55 Hz) in X or at δ 163.7 (J = 58 Hz) in XI, and the carbon at the 3-position as a doublet at δ 138.6 (J = 55 Hz) in X or at δ 133.4 (J = 58 Hz) in XI in the ^{13}C NMR spectra. 4-Hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XII) was obtained from

the bromo derivative (XI) and 1.5 equivalent moles of cuprous cyanide-^{13}C in dimethylformamide under reflux as a green yellow powder in 88% crude yield (58.5% crude yield based on cuprous cyanide-^{13}C). Cuprous cyanide-^{13}C was synthesized[5] from sodium cyanide-^{13}C (90 atom%) and cupric sulfate. The result of recrystallization of the product (XII) was not as good as expected, because of its low solubility in solvents. The product was therefore used for the next reaction without purification.

The carbonitrile-^{13}C (XII) was treated with 1.2 equivalent moles of ethyl iodide-1-^{13}C (90 atom%) using sodium hydride in dimethylformamide, giving 1-(ethyl-1-^{13}C)-6,7-methylenedioxy-4(1H)-oxocinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XIII) in 84.5% yield (70.5% yield based on ethyl iodide-1-^{13}C). The ^{13}C NMR spectrum showed a quartet at δ 165.9 (J = 53 and 11 Hz) due to the carbonyl carbon, an octet at δ 123.8 (J = 98, 53 and 3 Hz) due to the carbon at the 3-position, a quartet at δ 113.6 (J = 98 and 11 Hz) due to the carbonitrile carbon, and a doublet at δ 52.7 (J = 3 Hz) due to the N-methylenic carbon. Indirect ^{13}C-^{13}C couplings were found between the carbonyl and the carbonitrile carbon (^2J = 11 Hz) and between the carbon at the 3-position and the N-methylenic carbon (^3J = 3 Hz). The ^{13}C compound (XIII) was hydrolyzed with acetic acid and hydrochloric acid to give 1-(ethyl-1-^{13}C)-6,7-methylenedioxy-4(1H)-oxocinnoline-3,4-^{13}C-3-carboxylic acid-^{13}C (cinoxacin-^{13}C) (XV) as pale yellow crystals, m.p. 261~262° (dec.), in 38% yield. The ^{13}C NMR spectrum (d_6-DMSO) showed a quartet at δ 168.6 (J = 54 and 5 Hz) due to the carbon at the 4-position, a quartet at δ 162.9 (J = 78 and 5 Hz) due to the carboxyl carbon, an octet at δ 132.1 (J = 78, 54 and 3 Hz) due to the carbon at the 3-position, and a doublet at δ 53.4 (J = 3 Hz) for the N-methylenic carbon. Indirect ^{13}C-^{13}C couplings were also found between the carbon at the 4-position and the carboxylic carbon (^2J = 5 Hz) and between the carbon at the 3-position and the N-methylenic carbon (^3J = 3 Hz) (see Fig. 1).

The mass spectrum of cinoxacin-^{13}C (XV) showed the parent ions, M^+ 266 and 265, at a ratio of 71 : 29 (the ^{12}C compound, M^+ 262), and ion peaks at m/e 221 (the ^{12}C compound, 218) corresponding to the loss of CO_2-^{13}C and at m/e 45 due to CO_2-^{13}C. The fragment ion corresponding to the loss of CO_2 and the ethyl at N-1 was observed at m/e 192 for the ^{13}C

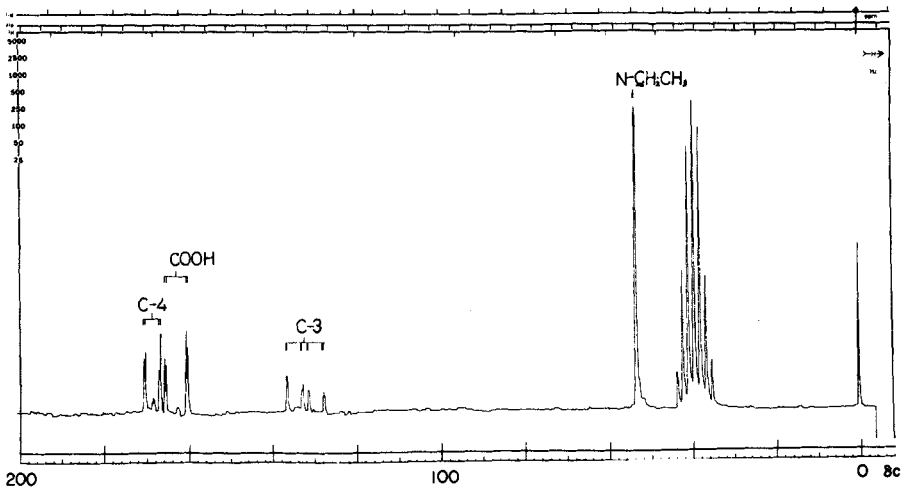


Fig. 1. The ^{13}C FT NMR spectrum of cinoxacin-^{13}C (XV) in d_6-DMSO at 15 MHz: number of transients, 71,000

compound (XV), but at m/e 190 for the ^{12}C compound (I). Moreover, the fragment ion (XVI) was observed at m/e 149 for XV and at m/e 148 for I. These results confirmed that the carbon-13 label was incorporated into the 3- and 4-positions of the cinnoline ring, the carboxyl group at C-3, and the methylene of the ethyl group at N-1 in cinoxacin (see Fig. 2).

Use of mass spectrometric techniques in biotransformation studies makes profitable use of deuterio- or ^{15}N-labelled compounds in such studies, if deuterium or ^{15}N is stable to metabolic and chemical loss. We tried labelling the methylenedioxy group with deuterium and the 2-position of the cinnoline ring with ^{15}N.

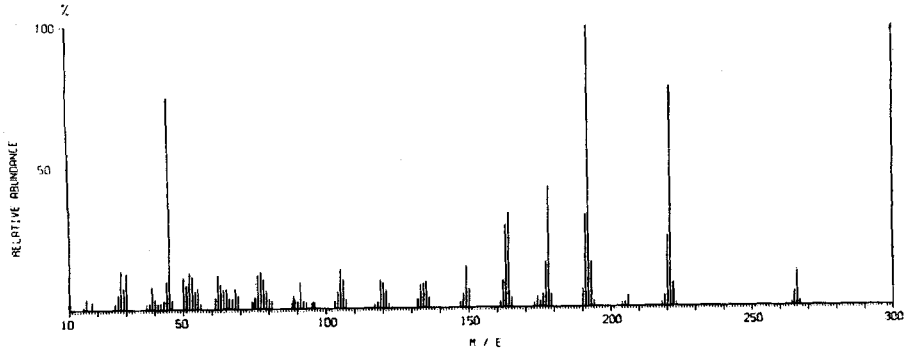


Fig. 2. The mass spectrum of cinoxacin-^{13}C

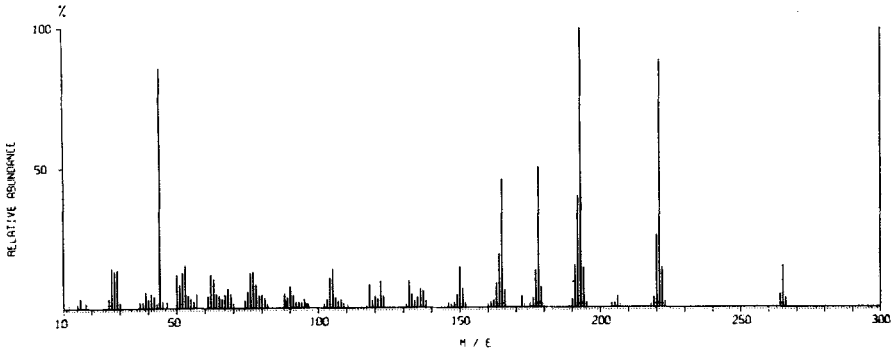


Fig. 3. The mass spectrum of cinoxacin-d, ^{15}N

1,2-Methylene-d_2-dioxybenzene was obtained from methylene-d_2-dichloride (99% isotopic purity, M.S.D. Co.) and O-dihydroxybenzene as a colourless oil, b.p. 108° / 100 mm, in 24.8% yield based on methylene-d_2-dichloride. Its mass spectrum showed the parent ion, M^+ 124, and the fragment ion, $(M-D)^+$ 122. Bromination of 1,2-methylene-d_2-dioxybenzene with NBS gave 3,4-methylene-d_2-dioxybromobenzene (II) a colourless oil, b.p. 86-87° /3 mm, in 66% yield. The deuterio compound (II) was carboxylated using dry ice in the same manner as the ^{14}C-carboxylation to give 3,4-methylene-d_2-dioxybenzoic acid (III), m.p. 228-229° . The mass spectrum displayed M^+ 168 and $(M-D)^+$ 166 at a ratio of 100 : 93. However, the fragment ion, $(M-D)^+$, was not observed in the spectrum of 3',4'-methylene-d_2-dioxyacetophenone (VII) derived from the acid (III). 2'-Amino-4',5'-methylene-d_2-dioxyacetophenone (IX), M^+ 181, obtained from the acetophenone (VII) by nitration followed by hydrogenation was treated with 1,3-equivalent moles of sodium nitrite-^{15}N (96.1% isotopic purity, M.S.D. Co.) to give 4-hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N (X), M^+ 193, in 71% yield (54% yield based on sodium nitrite-^{15}N). In the same manner as for cinoxacin-^{13}C, we obtained 6,7-methylene-d_2-dioxy-4(1H)-oxocinnoline-3-carboxylic acid-2-^{15}N (cinoxacin-d, ^{15}N) (XVII) from X.

The mass spectrum of cinoxacin-d, ^{15}N (XVII) showed the parent ion M^+ 265 and an ion peak at m/e 221 corresponding to the loss of CO_2. The fragment ion corresponding to the

loss of CO_2 and the ethyl at N-1 was observed at m/e 193; and the fragment ion (XVI) was observed at m/e 150 for XVII, but at m/e 148 for the ^{12}C compound (I).

Moreover, cinoxacin-2-^{15}N* (XVIII) was also synthesized for metabolism studies. The results of pharmacological studies with these labelled compounds will be described in the following paper.

EXPERIMENTAL

The ^{13}C FT NMR spectra were recorded on a Varian NV-14 FT NMR spectrometer at 15.087 MHz using $CDCl_3$ solutions, unless otherwise noted, in 8 mm tubes at 30° . FT measurement conditions were as follows: spectral width, 3017; pulse flipping angle, 16°; acquisition time, 0.6 sec.; number of data points, 3,706.

3,4-Methylenedioxybenzoic acid-1-^{14}C (III)

A solution of 964 mg of butyl lithium in n-hexane (15% BuLi in n-hexane) was added dropwise to a solution of 3.25 g (16.2 mM) of 3,4-methylenedioxybromobenzene (II) in 25 ml of anhydrous ether in a vacuum manifold Grignard apparatus with stirring for 30 min. at -30~35° . Carbon dioxide-^{14}C derived from 1.35 g (50 mCi, 6.85 mM) of barium carbonate-^{14}C and 620 mg (3.15 mM) of a carrier, cold barium carbonate and 60% perchloric acid solution (10 ml) were induced into the above reaction mixture at -30° with stirring for 15 min. Unreacted carbon dioxide was collected by cooling with liquid nitrogen and again induced into the reaction mixture at -20°~5° . The reaction mixture was adjusted to pH 1.0 by addition of 6N H_2SO_4, during which time crystals were separated from the solution and filtered. The collected crystalline product (III) was washed with water. The filtrate was extracted with ether, and the ether extract then was extracted with 5% $NaHCO_3$. A carrier, cold (III, 100 mg) was added to the ether layer, and the mixture was extracted with 5% $NaHCO_3$. The combined $NaHCO_3$ solution was acidified with 6N H_2SO_4 to give the crystalline product (III). The combined crystalline product was washed with water and dried over P_2O_5 in vacuo at 80°, giving 3,4-methylene dioxybenzoic acid-1-^{14}C, colourless crystals, m.p. 228~229° , (III, 1.5

g, 44.2 mCi, specific activity : 29.4 μCi/mg) in 88.4% radiochemical yield based on barium carbonate-^{14}C.

3,4-Methylenedioxybenzyl alcohol-1-^{14}C (IV)

Lithium aluminium hydride (500 mg) was added to a solution of 1.5 g of 3,4-methylenedioxybenzoic acid-1-^{14}C (III, 44.2 mCi) in 45 ml of dry tetrahydrofuran with stirring in an ice bath, and stirred for 30 min. at the same temperature and 1 hr. at room temperature. To this mixture, 6N H_2SO_4 was added with stirring in an ice bath, and the mixture was extracted with ether. The ether extract was washed with $NaHCO_3$ solution and NaCl solution, dried (Na_2SO_4), and evaporated, leaving a white solid (1.3 g), 3,4-methylenedioxybenzyl alcohol-1-^{14}C (IV, 42 mCi) in 95% radiochemical yield.

3,4-Methylenedioxybenzaldehyde-1-^{14}C (V)

A solution of 1.3 g of 3,4-methylenedioxybenzyl alcohol-1-^{14}C (IV, 42 mCi) in 5 ml of pyridine was added dropwise to chromium trioxide-pyridine complex (prepared from 2,6 g of chromium trioxide and 40 ml of pyridine) with stirring in an ice bath and left standing for 18 hr. at room temperature. A carrier, cold (V, 200 mg) was added to the reaction mixture, and the mixture was then poured into ice water (200 ml). The mixture was extracted with ether, washed with 6N H_2SO_4 and NaCl solution, dried (Na_2SO_4), and evaporated, leaving 3,4-methylenedioxybenzaldehyde-1-^{14}C (V), colorless crystals, m.p. 36~37° (1.3 g, 36.2 mCi) in 86.2% radiochemical yield.

1'-Hydroxyethyl-3,4-methylenedioxybenzene-1'-^{14}C (VI)

Grignard reagent was prepared from 2.55 g of methyl iodide and 410 mg of magnesium in 30 ml of anhydrous ether in a vacuum manifold Grignard apparatus. A solution of 1.3 g of 3,4-methylenedioxybenzaldehyde-1-^{14}C (V, 36.2 mCi) in 10 ml of anhydrous ether was added dropwise to the Grignard reagent at 0~10° with stirring and then stirred for 1 hr. at room temperature. A solution of NH_4Cl (10 g) in water (50 ml) was added dropwise to the reaction mixture with stirring and stirred for 15 min. in an ice bath. This mixture was extracted with ether, washed with water, dried (Na_2SO_4), and evaporated, leaving 1'-hydroxyethyl-3,4-

methylenedioxybenzene-1'-^{14}C (VI), an oil (1.44 g, 36.2 mCi) in a quantitative yield.

3',4'-Methylenedioxyacetophenone-1-^{14}C (VII)

A solution of 1.44 g of 1'-hydroxyethyl-3,4-methylenedioxybenzene-1'-^{14}C (VI, 36.2 mCi) in 5 ml of pyridine was added dropwise to chromium trioxide-pyridine complex (prepared from 3.0 g of chromium trioxide and 45 ml of pyridine) with stirring in an ice bath and left standing for 18 hr. at room temperature. The reaction mixture was poured into ice water (200 ml) and extracted with ether. The ether extract was washed with 6N H_2SO_4 and water, dried (Na_2SO_4), and evaporated, leaving a crystalline residue (1.46 g), to which a carrier, cold 3',4'-methylenedioxyacetophenone (200 mg) was added. The residue was recrystallized from dichloromethane-ether to give 3',4'-methylenedioxyacetophenone-1-^{14}C (VII), colourless prisms, m.p. 85~86° (1.37 g, 29.0 mCi) in 80% radiochemical yield.

2'-Nitro-4',5'-methylenedioxyacetophenone-1-^{14}C (VIII)

3',4'-Methylenedioxyacetophenone-1-^{14}C (VII, 1.37 g, 29.0 mCi) was added in a small portion to 15 ml of HNO_3 (d = 1.42) with stirring and stirred for 1 hr. in an ice bath. The mixture was poured into ice water (100 ml), extracted with dichloromethane, washed with water, dried (Na_2SO_4), and evaporated, leaving a residue (1.6 g). The residue was recrystallized from dichloromethane-ether to give 2'-nitro-4',5'-methylenedioxyacetophenone-1-^{14}C (VIII), colourless prisms (1.02 g), m.p. 123~124° . The mother liquor was evaporated to leave an oil (580 mg), which was chromatographed on silica gel (Merck KG-60, 10 g) to give prisms (VIII, 220 mg). The combined crystals (1.24 g) had 20.3 mCi of total activity, 3.42 mCi/mM of specific activity (radiochemical yield : 70.0%).

2'-Amino-4',5'-methylenedioxyacetophenone-1-^{14}C (IX)

Platinium dioxide (300 mg) was added to a solution of 2'-nitro-4',5'-methylenedioxyacetophenone-1-^{14}C (VIII, 1.24 g, 20.3 mCi) in ethanol (110 ml), and the mixture was hydrogenated at 13° until 520 ml of hydrogen was absorbed and filtered off. The filtrate was evaporated to leave a crystalline residue, which was recrystallized from dichloromethane-ether to give 2'-amino-4',5'-methylenedioxyacetophenone-1-^{14}C (IX), colourless needles,

m.p. 167~168° (984 mg, 19.0 mCi) in 93.5% radiochemical yield.

4-Hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (X)

2'-Amino-4',5'-methylenedioxyacetophenone-1-^{14}C (IX, 984 mg, 19.0 mCi) was added to conc. HCl (12 ml) in an ice bath. A solution of $NaNO_2$ (500 mg) in water (2 ml) was added dropwise to the solution with stirring at 0° for 1 hr. The reaction mixture was filtered, and the filtrate was warmed at 80° for 4 hr. and then allowed to cool in an ice bath to effect crystallisation of the product. The filtered crystals were washed with ice water (10 ml) and dried for 2 hr. at 80°, giving 4-hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (X), an ocherous powder (900 mg, 16.38 mCi) in 86.2% radiochemical yield.

3-Bromo-4-hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (XI)

A solution of 720 mg of bromine in 2 ml of acetic acid was added dropwise to a suspension of 500 mg of potassium acetate in a solution of 4-hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (X, 900 mg, 16.38 mCi) in acetic acid (6.2 ml) with stirring under reflux and stirred for an additional 30 min. The reaction mixture was allowed to cool to room temperature and then poured into 20 ml of ice water. The resultant precipitate was filtered, washed with cold water (10 ml), and dried for 2.5 hr. at 80°, giving 3-bromo-4-hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (XI), a white amorphous powder (1.15 g, 14.6 mCi) in 89.6% radiochemical yield.

4-Hydroxy-6,7-methylenedioxycinnoline-3-carbonitrile-4-^{14}C (XII)

3-Bromo-4-hydroxy-6,7-methylenedioxycinnoline-4-^{14}C (XI, 1.15 g, 14.6 mCi) was added to a solution of cuprous cyanide (730 mg) in dry dimethylformamide (13 ml) and heated under reflux for 4 hr. with stirring. To this mixture was added a solution of ferric chloride (1.81 g) in conc. HCl (1.4 ml) and water (3 ml). The mixture was heated at 60° for 25 min. and poured into 50 ml of ice water to separate a precipitate from the mixture. The precipitate was filtered, washed with cold water, dried for 2.5 hr. at 80°, giving 4-hydroxy-6,7-methylenedioxycinnoline-3-carbonitrile-4-^{14}C (XII), a yellow green powder (860 mg, 13.6 mCi) in 93.5% radiochemical yield.

1-Ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carbonitrile-4-^{14}C (XIII)

4-Hydroxy-6,7-methylenedioxycinnoline-3-carbonitrile-4-^{14}C (XII, 860 mg, 13.6 mCi) was suspended in dry dimethylformamide (8 ml) with stirring. Sodium hydride (113 mg) was added to the suspension with stirring at 10° and stirred for 10 min. at room temperature, during which time the mixture turned into a dark brown solution. Ethyl iodide (800 mg) was added dropwise to the solution with stirring and stirred at room temperature for 25 min. and then at 95~100° for 2 hr. The solution was poured into ice water (30 ml) and adjusted to pH 1.0 by addition of 10% HCl. The precipitate was collected, washed with water, and dried for 3 hr. at 80°, giving 1-ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carbonitrile-4-^{14}C (XIII), a yellow brown powder (865 mg, 12.1 mCi) in 89% radiochemical yield.

Cinoxacin-^{14}C (XIV)

1-Ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-3-carbonitrile-4-^{14}C (XIII, 865 mg, 12.1 mCi) was added to a solution of conc. HCl (13 ml) and acetic acid (13 ml) and heated under reflux for 3 hr. A solution (4 ml) of conc. HCl and acetic acid (1 : 1) was added to the mixture and heated under reflux for 2.5 hr. The acids were evaporated in vacuo to leave a residue, which was dissolved in 70 ml of 5% $NaHCO_3$ and filtered. The filtrate was extracted with chloroform. The aqueous layer was treated with 20 mg of charcoal and filtered. The filtrate was adjusted to pH 1.0 by addition of conc. HCl and the resulting precipitate was collected, washed with water, and dried for 2 hr. at 80°, giving a pale yellow powder (439 mg, 5.7 mCi). The residue was crystallized from chloroform-methanol (5 : 1) to give cinoxacin-^{14}C (XIV) as pale yellow crystals m.p. 261~262° (dec.) (385 mg, 5.0 mCi, specific activity : 3.42 mCi/mM) in 41.3% radiochemical yield (10.0% over-all yield based on barium carbonate-^{14}C). This compound was confirmed to be pure by t.l.c.-autoradiogram and t.l.c.-radioactinogram [X-ray film, silica gel KGF plate, solvent system = chloroform-methanol (7 : 3)].

3,4-Methylenedioxybenzoic acid-1-^{13}C (III)

3,4-Methylenedioxybenzoic acid-1-^{13}C (III) was obtained from 1.98 g of barium carbon-

ate-^{13}C [enriched with 90.7 atom% C-13 (M.S.D. Co.)] and 3.24 g of 3,4-methylenedioxybromobenzene (II) by use of 960 mg of butyl lithium as colourless crystals (1.2 g), m.p. 229° in 72% yield.

3,4-Methylenedioxybenzyl alcohol-1-^{13}C (IV)

Reduction of 5.1 g of 3,4-methylenedioxybenzoic acid-1-^{13}C (III) with 1.7 g of lithium aluminium hydride gave 3,4-methylenedioxybenzyl alcohol-1-^{13}C (IV) as a white solid (4.5 g) in 96.5% yield.

3,4-Methylenedioxybenzaldehyde-1-^{13}C (V)

3,4-Methylenedioxybenzyl alcohol-1-^{13}C (IV) (4.4 g) was oxidized with 8.0 g of chromium trioxide and 120 ml of pyridine to give 3,4-methylenedioxybenzaldehyde-1-^{13}C (V) as colourless crystals (3.9 g), m.p. 36~37° in 90% yield.

1'-Hydroxyethyl-3,4-methylenedioxybenzene-1',2'-^{13}C (VI)

Grignard reagent was prepared from 5.0 g (1.35 equiv. mol.) of methyl iodide-^{13}C [enriched with 90 atom% C-13 (M.S.D. Co.)] and 1 g of magnesium in 60 ml of anhydrous ether. Reaction of 3.9 g of 3,4-methylenedioxybenzaldehyde-1-^{13}C (V) with Grignard reagent gave 1'-hydroxyethyl-3,4-methylenedioxybenzene-1',2'-^{13}C (VI) as a colourless oil (4.3 g) in 99% yield.

3',4'-Methylenedioxyacetophenone-1,2-^{13}C (VII)

A solution of 4.2 g of 1'-hydroxyethyl-3,4-methylenedioxybenzene-1',2'-^{13}C (VI) in 10 ml of pyridine was oxidized with 10 g of chromium trioxide and 140 ml of pyridine at room temperature for 18 hr. 3',4'-Methylenedioxyacetophenone-1,2-^{13}C (VII) was obtained as colourless prisms, m.p. 85~86° (3.9 g) in 94% yield.

2'-Nitro-4',5'-methylenedioxyacetophenone-1,2-^{13}C (VIII)

3',4'-Methylenedioxyacetophenone-1,2-^{13}C (VII, 3.9 g) was added to 40 ml of HNO_3 (d = 1.42) with stirring in an ice bath. 2'-Nitro-4',5'-methylenedioxyacetophenone-1,2-^{13}C (VIII) was obtained as colourless prisms, m.p. 123~124° (3.5 g) (from dichloromethane-

ether) in 71% yield.

2'-Amino-4',5'-methylenedioxyacetophenone-1,2-^{13}C (IX)

A solution of 3.5 g of 2'-nitro-4',5'-methylenedioxyacetophenone-1,2-^{13}C (VIII) in 320 ml of ethanol was hydrogenated with 850 mg of platinium dioxide as a catalyst, until 1460 ml of hydrogen was absorbed. 2'-Amino-4',5'-methylenedioxyacetophenone-1,2-^{13}C (IX) was obtained as colourless needles, m.p. 169~171° (2.7 g) (from dichloromethane-ether) in 90% yield.

4-Hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (X)

A solution of 1.35 g of $NaNO_2$ in 5 ml of water was added to a solution of 2.7 g of 2'-amino-4',5'-methylenedioxyacetophenone-1,2-^{13}C (IX) in 33 ml of conc. HCl in an ice bath, and the reaction mixture was heated at 80° for 4 hr. 4-Hydroxy-6,7-methylenedioxy-cinnoline-3,4-^{13}C (X) was obtained as an ocherous powder (2.5 g) in 87% yield.

3-Bromo-4-hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (XI)

A mixture of 4-hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (X, 2.5 g) and potassium acetate (1.27 g) in 17 ml of acetic acid was brominated with a solution of 2 g of bromine in 5 ml of acetic acid to give 3-bromo-4-hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (XI) as a white powder (2.8 g) in 79.5% yield.

4-Hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XII)

Cuprous cyanide-^{13}C (850 mg) was obtained[5] from 1 g of sodium cyanide-^{13}C [enriched with 90 atom% C-13 (M.S.D. Co.)] and 1.6 g of cupric sulfate. A mixture of 3-bromo-4-hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C (XI, 1.7 g) and cuprous cyanide-^{13}C (850 mg, 1.5 equiv. mol.) in 20 ml of dry dimethylformamide was heated under reflux for 4 hr. to give 4-hydroxy-6,7-methylenedioxycinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XII) as a yellow green powder (1.2 g) in 88% yield (58.5% yield based on cuprous cyanide-^{13}C).

1-(Ethyl-1-^{13}C)-6,7-methylenedioxy-4(1H)-oxocinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XIII)

Ethyl iodide-1-^{13}C [enriched with 90 atom% C-13 (M.S.D. Co.)] (1 g, 1.2 equiv. mol.)

was added to a brown solution obtained from a suspension of 4-hydroxy-6,7-methylenedioxy-cinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XII, 1.15 g) in dry dimethylformamide (10 ml) by addition of sodium hydride (154 mg), and heated at 95~100° for 2 hr., giving 1-(ethyl-1-^{13}C)-6,7-methylenedioxy-4(1H)-oxocinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XIII) as a yellow brown powder (1.1 g) in 84.5% yield (70% yield based on ethyl iodide-1-^{13}C).

1-(Ethyl-1-^{13}C)-6,7-methylenedioxy-4(1H)-oxocinnoline-3,4-^{13}C-3-carboxylic acid-^{13}C (cinoxacin-^{13}C) (XV)

1-(Ethyl-1-^{13}C)-6,7-methylenedioxy-4(1H)-oxocinnoline-3,4-^{13}C-3-carbonitrile-^{13}C (XIII, 1.05 g) was hydrolyzed with a solution (30 ml) of conc. HCl and acetic acid (1 : 1) to give cinoxacin-^{13}C (XV) as pale yellow crystals (from chloroform-methanol), m.p. 261~263° (dec.) (430 mg) in 38% yield.

1,2-Methylene-d_2-dioxybenzene

Sixty eight grams of o-dihydroxybenzene was added to a solution of 46 g of NaOH in 320 ml of 95% ethanol and 21 ml of water. This solution and 68 g of methylene-d_2-dichloride (99% isotope purity, M.S.D. Co.) were added to an autoclave (1000 ml) and stirred for 17 hr. at 110~120°. The mixture was poured into 2 l of ice water and extracted with ether. The ether extract was washed with 5% NaOH and water, dried (Na_2SO_4), and evaporated, leaving a residue, to which ether (20~30 ml) was added and left standing to separate crystals (by-product). The mixture was filtered off and the filtrate was distilled to give 1,2-methylene-d_2-dioxybenzene as a colourless oil, b.p. 108°/100 mm (24.5 g) in 24.8% yield based on methylene-d_2-dichloride.

3,4-Methylene-d_2-dioxybromobenzene (II)

1,2-Methylene-d_2-dioxybenzene (24 g) and N-bromosuccinimide (37.4 g) were added to chloroform (105 ml) and heated under reflux for 3.5 hr. The mixture was then allowed to cool to separate crystals from the solution, and filtered off. The crystals were washed with cold chloroform. The filtrate and the washings were combined, washed with water, dried (Na_2SO_4), and evaporated, leaving an oily residue. The residue was distilled to give 3,4-

methylene-d_2-dioxybromobenzene (II) as an oil, b.p. 86~87°/3 mm (26 g) in 66% yield.

3,4-Methylene-d_2-dioxybenzoic acid (III)

A solution of butyl lithium (1.66 g) in n-hexane (16.6 ml) was added dropwise to a solution of 3,4-methylene-d_2-dioxybromobenzene (II, 4 g) in anhydrous ether (80 ml) with stirring for 20 min. at -30°. Dry ice (1.7 g) was added to the mixture with stirring at -30° and stirred for 1 hr. at -30°~ 0°. 3,4-Methylene-d_2-dioxybenzoic acid (III) was obtained as colourless crystals (1.5 g) in 45.5% yield.

3,4-Methylene-d_2-dioxybenzyl alcohol (IV)

Reduction of III (7 g) with lithium aluminium hydride (2.3 g) gave 3,4-methylene-d_2-dioxybenzyl alcohol (IV), a white solid (6.3 g) in 98% yield.

3,4-Methylene-d_2-dioxybenzaldehyde (V)

Oxidation of IV (6.5 g) with chromium trioxide (6.5 g) and pyridine (200 ml) gave 3,4-methylene-d_2-dioxybenzaldehyde (V), colourless crystals (5 g) in 78% yield.

1'-Hydroxyethyl-3,4-methylene-d_2-dioxybenzene (VI)

Grignard reaction of V (4.7 g) with Grignard reagent obtained from methyl iodide (8.75 g) and magnesium (1.44 g) in anhydrous ether (100 ml) gave 1'-hydroxyethyl-3,4-methylene-d_2-dioxybenzene (VI), a colourless oil (4.8 g) in 90.5% yield.

3',4'-Methylene-d_2-dioxyacetophenone (VII)

Oxidation of VI (4.8 g) with chromium trioxide (12 g) and pyridine (150 ml) gave 3',4'-methylene-d_2-dioxyacetophenone (VII), colourless prisms, m.p. 85~86° (4.2 g) in 88% yield.

2'-Nitro-4',5'-methylene-d_2-dioxyacetophenone (VIII)

Nitration of VII (4.2 g) with HNO_3 (d = 1.42) (50 ml) gave 2'-nitro-4',5'-methylene-d_2-dioxyacetophenone (VIII), colourless prisms, m.p. 123~124° (3.86 g) in 72% yield.

2'-Amino-4',5'-methylene-d_2-dioxyacetophenone (IX)

A solution of VIII (3.13 g) in 99% ethanol (250 ml) was hydrogenated with platinium

dioxide (700 mg) as a catalyst to give 2'-amino-4',5'-methylene-d_2-dioxyacetophenone (IX), colourless needles, m.p. 169~171° (2.2 g) in 82% yield.

4-Hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N (X)

Treatment of a solution of IX (1.8 g) in conc. HCl (20 ml) with $NaNO_2$-^{15}N (96.1% isotope purity, M.S.D. Co.) (900 mg, 1.3 equiv. mol.) gave 4-hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N (X), an ocherous powder (1.36 g) in 71% yield (54% yield based on $NaNO_2$-^{15}N).

3-Bromo-4-hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N (XI)

Bromination of X (1.3 g) with bromine (1.08 g) gave 3-bromo-4-hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N (XI), an amorphous powder (1.69 g) in 92% yield.

4-Hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N-3-carbonitrile (XII)

Treatment of XI (1.64 g) and cuprous cyanide (1.17 g) in dry dimethylformamide (20 ml) gave 4-hydroxy-6,7-methylene-d_2-dioxycinnoline-2-^{15}N-3-carbonitrile (XII), a yellow green powder (1.12 g) in 85% yield.

1-Ethyl-6,7-methylene-d_2-dioxy-4(1H)-oxocinnoline-2-^{15}N-3-carbonitrile (XIII)

Ethylation of XII (1.05 g) was performed with ethyl iodide (910 mg, 1.2 equiv. mol.) and sodium hydride (117 mg) in dry dimethylformamide (10 ml), giving 1-ethyl-6,7-methylene-d_2-dioxy-4(1H)-oxocinnoline-2-^{15}N-3-carbonitrile (XIII), a yellow brown powder (940 mg) in 79.5% yield.

1-Ethyl-6,7-methylene-d_2-dioxy-4(1H)-oxocinnoline-2-^{15}N-3-carboxylic acid (cinoxacin-d, ^{15}N) (XVII)

Hydrolysis of XIII (940 mg) with a solution (27 ml) of conc. HCl and acetic acid (1 : 1) gave 1-ethyl-6,7-methylene-d_2-dioxy-4(1H)-oxocinnoline-2-^{15}N-carboxylic acid (XVII), pale yellow crystals, m.p. 262~263° (dec.) (500 mg) in 49.3% yield.

1-Ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-2-^{15}N-3-carboxylic acid (cinoxacin-^{15}N)* (XVIII)

2'-Amino-4',5'-methylenedioxyacetophenone (IX, 2 g) was treated with $NaNO_2$-^{15}N (96.1% isotope purity, M.S.D. Co.) (1 g, 1.28 equiv. mol.) to give 4-hydroxy-6,7-methylenedioxycinnoline-2-^{15}N (X, 1.54 g, 72.5% yield), form which 1-ethyl-6,7-methylenedioxy-4(1H)-oxocinnoline-2-^{15}N-3-carboxylic acid (*cinoxacin-^{15}N*) (XVIII) was obtained as pale yellow crystals, m.p. 261~263° (dec.) (630 mg) in the same manner as in the case of cinoxacin-^{14}C (XIV).

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