SYNTHESIS OF CARBON-14 AND DEUTERIUM LABELED 3-ISOBUTYRYL-2-ISOPROPYLPYRAZOLO[1,5-a]PYRIDINE

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SUMMARY

In order to study the metabolic fate of 3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine (I, KC-404: antiasthmatic agent) in experimental animals and humans, the synthesis of I labeled with carbon-14 or deuterium was investigated. Carbon-14 labeled I was obtained from 5 mCi of isobutyric acid-1-^{14}C sodium salt with a 67.7% yield. Its specific activity was 24.7 μCi/mg. Deuterium labeled I was synthesized from pyridine-d_5 with a 17.8% yield, which resulted in about 50% replacement of deuterium with hydrogen at the position 7 in I. The release of deuterium apparently did not occur on GC-MS. The mixture of deuterium labeled I and unlabeled I (2:1 mole ratio) supplied nearly the same intensity of triplet ions (ion clusters) used for identification of I and its metabolites.

Key Words: antiasthmatic agent, carbon-14, deuterium, ion cluster

INTRODUCTION

3-Isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine (KC-404) has various pharmaceutical activities such as dilation of tracheal or peripheral blood vessels and increasing of cerebral blood flow.[1),2)] KC-404 was developed as an antiasthmatic agent and for the enhancement of cerebral blood circulation; general and specific toxicity

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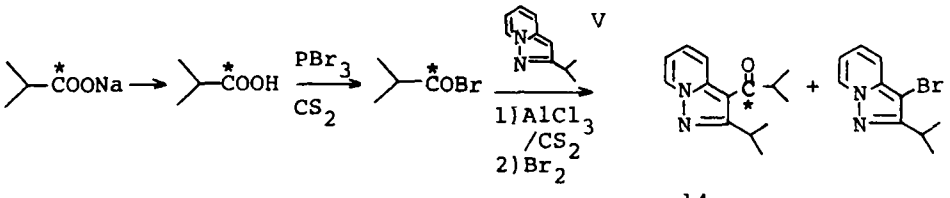
studies showed the agent to have very high security.[3]

Carbon-14 and deuterium labeled KC-404 were synthesized in order to study the metabolic fate of KC-404 in several experimental animals and humans. Preliminary small scale experiments were carried out to determine a suitable position of the carbon-14 labeling relative to the commercial availability of the starting materials, the facility of performing the synthesis, and stability of label in metabolic pathways. KC-404 labeled with carbon-14 in the carbonyl moiety of isobutyryl radical was orally administered in the rat. We found that the excretion of ^{14}C in air as $^{14}CO_2$ was only 0.05% of the total dose over a 24 hr period. It was therefore seemed valid to use the ^{14}C-KC-404 labeled at the carbonyl of the isobutyryl radical for the present metabolic studies.

Multiple-deuterium labeled compounds were commercially available as solvents for NMR. They were also low in price, readily obtainable and highly enriched in deuterium as clearly detected from the peaks of $(M+1)^+$ and $(M+2)^+$ ion. However, there are several problems in using multiple-deuterium labeled compounds, such as a biological isotope effect, elimination of deuterium in preparation or metabolism, deuterium-hydrogen exchange reaction in the gas chromatograph and mass spectrometer.[4),5)] In this report, we describe the synthesis of carbon-14 and deuterium labeled KC-404 and our fundamental investigations on detection of the metabolites of KC-404 by the ion cluster technique on GC-MS.

DISCUSSION

The synthetic route of ^{14}C-KC-404 is illustrated in Chart 1. By reaction of equimolar IV and V in the cold run, I was prepared at a yield of 52-53%. Considerable amounts of V remained in the reaction mixture. Because it was difficult to separate I from V, V was



II III IV I (^{14}C-KC-404) VI

$\overset{*}{C}=^{14}C$

Chart 1

brominated to VI. By this procedure, product I was easily isolated and purified by column chromatography. Accordingly as shown in Fig. 1, VI was eluted with hexane at 10 times volume on an aluminum oxide column. I was then eluted with a mixture solvent of hexane-C_6H_6 (1:1 v/v) of about 10 times volume on aluminum oxide.

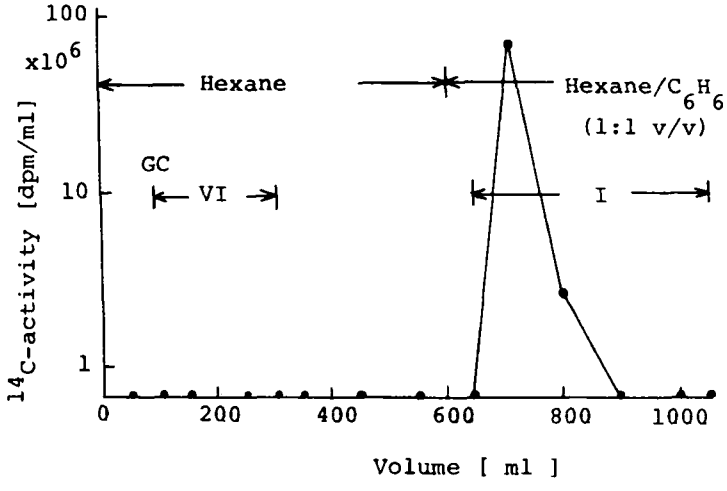


Fig. 1 Elution of ^{14}C-KC-404 (I) On Al_2O_3 column

The radiochemical purity of I was 99% as determined by reverse dilution analysis and TLC. Its specific activity was 24.7 μCi/mg; the I was therefore considered suitable for use as a tracer at a dose of

po 2.0 mg/kg and iv 0.5 mg/kg in experimental animals. The synthetic route of d_4-KC-404 is illustrated in Chart 2.

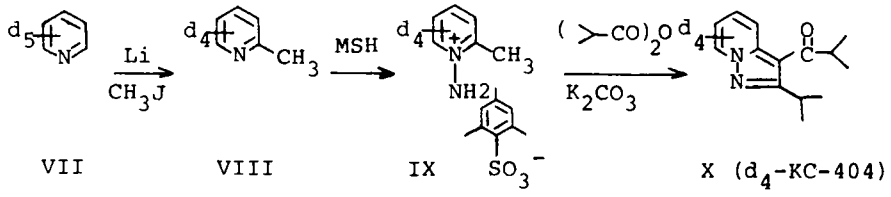


Chart 2

In accordance with the method of Ziegler and Zeiser[6], α-picoline -d_4 (VIII, arom.d_4) was obtained from pyridine-d_5 (VII, arom.d_5). VIII was N-aminated to IX with o-mesitylenesulfonylhydroxylamine (MSH) that was synthesized by the method of Tamura et al.[7] By reaction of IX and isobutyric anhydride, X was obtained at a 32.3% yield from VIII. Product X was a mixture of d_4 and d_3 labeled I as shown in Fig. 2.

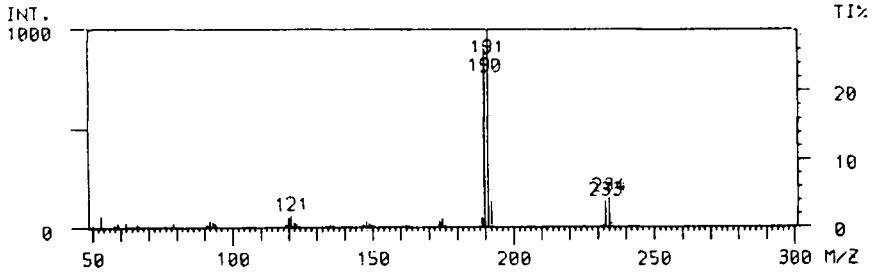


Fig. 2 Mass spectrum of X

d_3-Labeled I indicated a singlet peak at 8.48 ppm in a NMR spectrum. Deuterium was lacking at position 7 of the ring. There was no elimination of deuterium of VIII, as shown in a mass spectrum. It was therefore supposed that deuterium was released during the N-amination of VIII or ring close reaction of IX to X. The elimination of deuterium probably did not occur in the latter reaction. [8] Deuterium might have been released at N-amination of VIII to IX with MSH. The mechanism of this reaction was believed to result from the addition of nitrene from MSH to nitrogen of picoline. However, it was estimated as a 1,6-addition of MSH to double bond with the elimination of mesitylenesulfonic acid as shown in Chart 3.

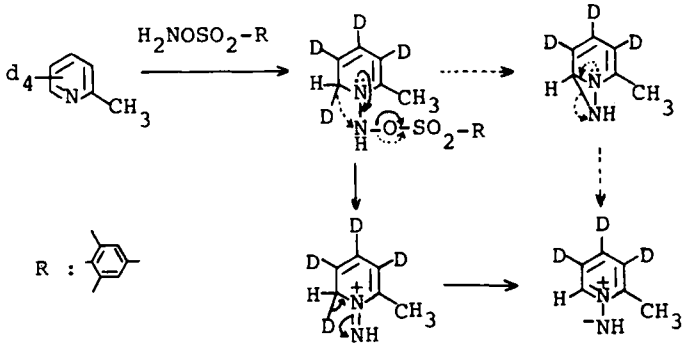


Chart 3

Because the ratio of d_4 and d_3 labeled product was 1:1, isotope effect was not observed in the reaction. The mixture of X and KC-404 (2:1 mole ratio) gave a specific triplet ion (ion cluster) having 3-4 m/z difference in the mass spectrum, as illustrated in Fig. 3. Using these ion clusters as a marker, the metabolite of KC-404 is readily detectable in the mass spectrum. The position and intervals of ion cluster also provide solid evidence on the chemical structure

of metabolites.

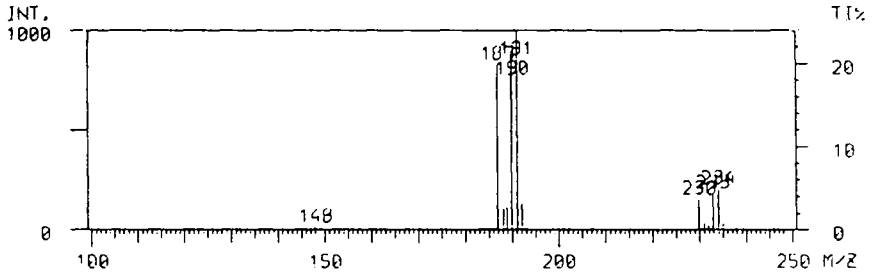


Fig. 3 Mass spectrum of the mixture of X and KC-404 (2:1 mole ratio)

EXPERIMENTAL

Measurement

GC was measured on GC-6A (Shimadzu), using a glass column (3 mm ϕ x 1 m) packed with 5% OV-1 Chromosorb WAW (60-80 mesh). Helium was used as the carrier gas (40 ml/min). The injection and column temperature were 180° and 150°, respectively. Radioactivity was determined on a Liquid Scintillation Spectrometer Tri-Carb 2425 (Packard) correcting the quenching by the external standard ratio method. TLC was developed on Kieselgel 60 F_{254} plate (0.25 mm, Merck) and a radiochromatogram was obtained using a Radiochromato Scanner 7201 (Packard). Mass spectrum was obtained by a Jeol GC-MS-COM equipped with JGC-20K, JMS-D300 and JMA-3100 computer. GC was made on a glass column (2 mmϕ x 1 m) packed with 3% OV-61 Gaschrom Q (80-100 mesh). The injection temperature was 270° and the column was 200-300° (5°/min). The electron energy was set to 24 eV. Scan speed was set to 3-5 second. NMR was measured in $CDCl_3$ with tetramethylsilane as the internal standard on FX 90Q (Jeol).

3-Isobutyryl-[carbonyl-^{14}C]-2-isopropylpyrazolo[1,5-a]pyridine (I, ^{14}C-KC-404)

Isobutyric acid-1-^{14}C sodium salt (II, 50 mCi/mmole, CEA, France) and unlabeled II (99.1 mg) were dissolved in 18 ml of water. Four ml of conc. hydrochloric acid was added and then the mixture was extracted with CH_2Cl_2. The extract was dried over anhydrous magnesium sulfate and allowed to evaporate at 20° to give 81.2 mg of III (92.2%). Three ml of CS_2 and 810 mg of PBr_3 were added to III and the mixture was refluxed for 1 hr at 80°. After the reaction mixture was cooled with an ice bath, 400 mg of $AlCl_3$ and 2 ml solution of CS_2 dissolved in 160 mg of V were added. The mixture was stirred for 23 hr at room temperature, and then was poured onto ice, extracted with $CHCl_3$. The $CHCl_3$ layer was washed with a 10% K_2CO_3 solution and rinsed with water. Four drops of Br_2 were added to the $CHCl_3$ layer cooled in an ice bath. After excess Br_2 was eliminated with a 10% $Na_2S_2O_3$ solution, the $CHCl_3$ layer was dried over anhydrous magnesium sulfate and condensed to give an oily residue. The residue was dissolved in a small volume of hexane and then applied to 30 ml of aluminum oxide on a column (90, Merck). The residue was then eluted with hexane at 17 times volume of aluminum oxide at 2 ml/min of flow rate, followed by hexane-C_6H_6 (1:1 v/v) at 14 times volume. Product VI, but not fraction I, produced a blue fluorescence when irradiated with long ultraviolet rays (3650°). We were thus able to adjust fraction volume to 50-100 ml by observation of this fluorescence. Ten ml of Aquasol-2 (NEN) was then added to 20-50 μl samples of each fraction and their radioactivities measured. Each fraction was next analyzed by GC. The fractions containing I were collected and evaporated to give 137.1 mg of I as a powder. The specific activity of I was 24.7 μCi/mg (5.69 mCi/mmole). The overall radiochemical yield of I from II was 67.7%. TLC-radiochro-

matograms (hexane/AcOH=9:1 v/v, Rf=0.35, $CHCl_3/C_6H_6$=2:1 v/v, Rf=0.42 , CH_2Cl_2/MeOH=20:1 v/v, Rf=0.92) were a single spot of ^{14}C-activity with more than 99% of radiochemical purity; GC retention time was 6.3 min.

Measurement of the reverse dilution analysis of I

A 1373086 dpm (total counts used; T) of I and 122.9 mg (weight used; W) of KC-404 were dissolved in ethanol and the solution was condensed by N_2 gas. The residue was dissolved in a small volume of hexane, applied to 20 ml of an aluminum oxide column (ø 1.5 cm) and washed with 300 ml of hexane. ^{14}C-KC-404 was then eluted with 100 ml of hexane-C_6H_6 (1:1 v/v), and its chemical purity was 99% as measured by GC. The specific activity of purified ^{14}C-KC-404 was 11229 dpm/mg (specific activity used; S). Therefore, the radiochemical purity of I was 100.5% (100 x W x S / T).

α-Picoline-d_4 (VIII)

A mixture of 31.3 g of methyl iodide, 3.34 g of lithium and 50 ml of ether was refluxed for 1 hr. To the mixture, 20 ml of C_6H_6 dissolved in 10 ml of pyridine-d_5 (VII, 99 atom%D, Merck Sharp and Dohme,Canada) was added. The mixture was then stirred for 30 min at room temperature and refluxed for 3 hr. The reaction mixture was poured onto ice, and then acidified with conc. hydrochloric acid. After removal of the ether and C_6H_6, the aqueous solution was saturated with sodium hydroxide and extracted with ether. The extract was concentrated to give 6.6 g of VIII as a colorless oil (55.0%); GC retention time was 4.0 min; MS M^+=97 m/z (93 atom%); NMR ppm ($CDCl_3$/TMS) 2.55 (singlet).

3-Isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine-d_4,d_3 (X)

A mixture of 2.0 g of VIII, 20 ml of CH_2Cl_2 and 7.7 g of o-mesitylenesulfonylhydroxylamine was stirred for 30 min at room temperature. The mixture was then condensed under reduced pressure. The residue was washed with ether and allowed to dry, giving 9.6 g of IX. Without further purification, IX was added to 25 ml of isobutyric anhydride and 2 g of K_2CO_3 and refluxed for 13 hr in an oil bath (160°). The reaction mixture was next evaporated. Water was then added, the mixture made basic with sodium hydroxide and finally extracted with CH_2Cl_2. The CH_2Cl_2 layer was condensed and then recrystallized from petroleum ether to give 1555 mg of X as colorless crystals. The overall yield from VIII was 32.3%; mp 56-57°; GC-MS retention time was 4.4 min; MS M^+=234, 233 m/z; NMR ppm ($CDCl_3$/TMS) 1.25 (6H, d), 1.40 (6H, d), 3.34 (1H, m), 3.79 (1H, m), 8.48 (1/2H, s).

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