

Synthesis of 3,4-Dimethylphenyl *N*-Methylcarbamate- ^{14}C (Meobal- ^{14}C) Masaaki Hazue and Kunio Miyake (Pharmaceuticals Division, Sumitomo Chemical Co., Ltd. Takarazuka, Hyogo, Japan) Received June 6, 1969. *Botyu-Kagaku*, 34, 120, 1969.

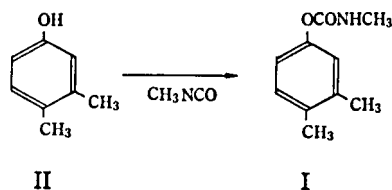
16. *N*-メチルカルバミン酸 3,4-ジメチルフェニル- ^{14}C (メオバル- ^{14}C) の合成 葉杖正昭・三宅邦男 (住友化学工業株式会社・医薬事業部・研究部) 44. 6. 6 受理

N-メチルカルバミン酸 3,4-ジメチルフェニル (メオバル) の代謝研究のために、3-メチル-4-メチル- ^{14}C フェノール および *N*-メチルカルバミン酸 3-メチル-4-メチル- ^{14}C フェニルを合成した。少量、放射性合成に適した合成経路、精製法などについて検討して次のような合成法を採用、好結果を得ることができた、3-メチル-4-ヨードアニソールと n -ブチルリチウムの金属交換反応によって3-メチル-4-リチウムアニソールを得、これにメタノール- ^{14}C から合成したヨウ化メチル- ^{14}C を作用させて、放射性ジメチルアニソールを得、つづいて、加水分解して3-メチル-4-メチル- ^{14}C フェノールを合成した。(収率：メタノール- ^{14}C より 72.5%) このキシレノール- ^{14}C にイソシアン酸メチルを作用させて93%収率で *N*-メチルカルバミン酸 3-メチル-4-メチル- ^{14}C フェニルを得た。合成物の融点、赤外吸収スペクトルは標準品とよく一致した。また 両標識化合物について薄層クロマトグラムの放射能走査、発色テストをおこない、化学的、放射化学的不純物を含まないことを確認した。

3,4-Dimethylphenyl *N*-methylcarbamate (Meobal, Sumitomo Chemical Co., I) and 3,4-xylenol (II) labelled with isotopic carbon were required for metabolic studies on 3,4-dimethylphenyl *N*-methylcarbamate¹⁾. The materials were to be specifically labelled at a site other than the *N*-methylcarbamyl position and the required specific radioactivity was one millicurie per millimole or more.

The labelled 3,4-dimethylphenyl *N*-methylcarbamate can be prepared *via* 3,4-xylenol- ^{14}C (scheme I)²⁾ and, since the ring labelled compound was not necessary for our purpose, a much simpler synthesis yielding the 3,4-xylenol- ^{14}C labelled at the methyl group was devised. The present paper deals with synthetic and purifying devices on a semimicro scale and radioactive preparation of 3,4-dimethylphenyl *N*-methylcarbamate.

scheme I



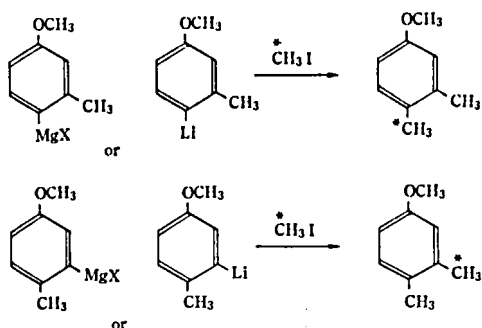
For the preparation of 3,4-xylenol-(methyl)- ^{14}C , several procedures were considered to be

available. One of the most common methods for introducing methyl- ^{14}C group into an aromatic ring is apparently a Grignard carbonation using radioactive carbon dioxide on an appropriate halogen compound followed by reduction of the resulting carboxyl group to methyl group³⁾. This method, however, is not proved consistently successful in obtaining the labelled 3,4-xylenol in high radiochemical yield, since the reduction of carboxyl group to methyl group must require two separated steps, i.e., from carboxylic acid to alcohol and from the alcohol to the methyl compound³⁾.

During the course of works, we were led to an attractive method of Biggerstaff and coworkers with which they obtained satisfactory results in the synthesis of *p*-cyclohexenylanisole⁴⁾. According to the method, the coupling reaction of an appropriate Grignard reagent or organolithium compound with methyl- ^{14}C iodide, the preparative method of which is well established⁵⁾, could yield the desired labelled dimethylanisole in one step (scheme II).

While these coupling reactions with alkyl halides were now well known, there are few examples for radioactive syntheses⁶⁾ and our first attempt to prepare the dimethylanisole using a Grignard reagent obtained from 3-methyl-4-iodoanisole was unsuccessful. The main reaction

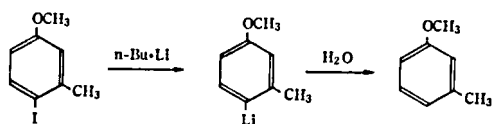
scheme II



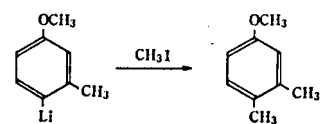
product was apparently not the expected 3,4-dimethylanisole but a hydrolysis product, 3-methylanisole. Thus, the direct ^{14}C -methylation was thoroughly explored on organo-lithium reagent which can be prepared from 3-methyl-4-iodoanisole by 1) the halogen replacement reaction; 2) the halogen-metal interconversion reaction. The former method was not successful and the halogen-metal interconversion reaction using *n*-butyllithium gave the intended lithium compound almost quantitatively with the only by-product, *n*-butyl iodide which was easily removed.

Amounts of methylanisole and 3,4-dimethylanisole formed in the reactions shown in schemes III and IV, respectively, were gas-chromatographically measured to determine the reaction conditions.

scheme III



scheme IV



Consequently, for the preparation of 3-methyl-4-lithioanisole, it was recognized to give the best result when the reaction mixture was refluxed for 5 min after addition of *n*-butyllithium (dissolved in *n*-hexane) into a boiled ethereal solution of 3-methyl-4-iodoanisole; the longer reaction time seemed rather to lower the yield

of 3-methylanisole, by-producing an impurity of high boiling point. At the lower reaction temperature (-20° and 0°C) the reaction was too slow to be useful; the yield of the product was low and not increased when the duration of reaction was varied from five minutes to two hours.

Regarding the methylation on the lithium reagent, the amount of 3,4-dimethylanisole reached maximal when the reaction mixture was refluxed for one hour after addition of the methyl iodide into the mixture containing the lithium methylanisole. Less amount of methyl- ^{14}C iodide than stoichiometrically required was added to make good use of it. Thus, in the reaction mixture after the methylation, considerable amounts of the excessively added reagents and a by-produced *n*-butyl iodide were contained. To remove these impurities, the reaction mixture after vacuum evaporation of solvent was submitted to a preparative gas-chromatography to separate pure 3,4-dimethylanisole. It was yielded almost quantitatively.

Several methods for the conversion of the dimethylanisole- ^{14}C to the corresponding phenol- ^{14}C were tried. The method described in the "Organic Syntheses"⁷⁾ showed to be the most promise, and it was modified to adapt to a small scale for use of a radioactive compound. The formation of *N*-methylcarbamate from the phenol was anticipated to present no difficulties since an adequate and well established method has been described. Thus the final step involving the addition reaction of methyl isocyanate with the dimethylphenol- ^{14}C was essentially that of Skrabas's procedure²⁾.

Radiochemical and chemical purity of the final product was assayed by radiochromatogram scanning and visualizing in silica gel H thin-layer which gave one radioactivity peak and one visualized spot corresponding to an R_f -value of 0.75 of an authentic sample.

Experimental

Materials: Methyl- ^{14}C alcohol having a specific radioactivity of 8 mCi/mmol, supplied from Japan Atomic Energy Research Institute, was used without any purifying treatment. 3-Methyl-4-iodoanisole prepared from commercially avail-

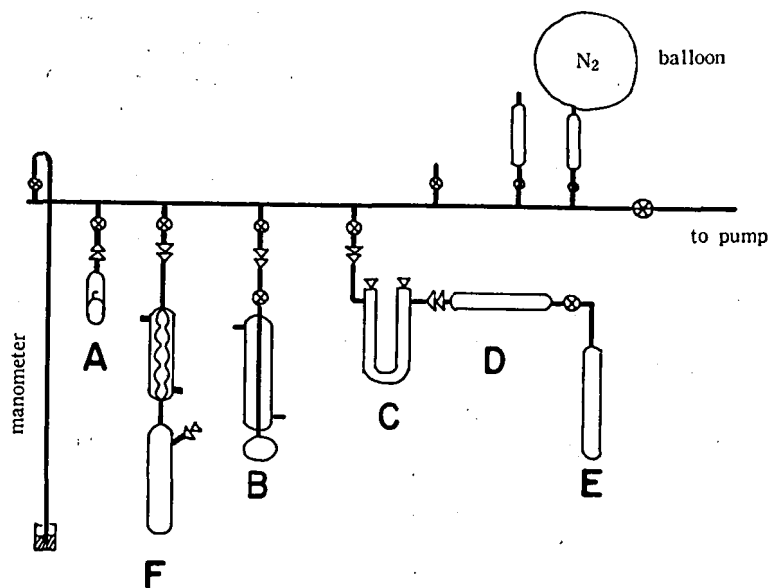


Fig. 1. Apparatus for 3,4-dimethyl-¹⁴C anisole preparation.

able *m*-cresol according to the direction of Sato and coworkers⁹ was used after recrystallization from ethanol; m. p.; 44–45°C. *n*-Hexane solution of *n*-butyllithium purchased from Nakarai Chemical Co., and assayed by duplicate titration using water and benzyl chloride to include 1.08 mmoles/ml of *n*-butyllithium was used.

Apparatus: Melting points were measured with "Yanagimoto Micro Melting Point Apparatus" and the corrected values are described. For measurements of radioactivity of products, liquid scintillation counting system (Kobe Kogyo, model GSL-163) was used, dissolving an aliquot of the products, into a liquid scintillator of the usual toluene-POPOP-*p*-terphenyl system. Radio-thin-layer chromatograms were scanned by "Aloka Thin-Layer Chromatography Scanner, model TLC-28". A high vacuum manifold equipped with a breakable sealed tube A containing methyl-¹⁴C alcohol, a reaction flask B, a purifying train (C and D) containing granulated sodalime and phosphorus pentoxide-on-glass wool, a product receiver E for methyl-¹⁴C iodide preparation, and a reaction flask F for dimethylanisole-¹⁴C preparation, was framed (Fig. 1). Reactions using the volatile isotopic materials were carried out thoroughly in this vacuum system.

Methyl-¹⁴C iodide: The method used here was virtually identical with that described in "Organic Syntheses with Isotopes"¹⁰. Thus, 100 μ l of non-radioactive methanol and 8 ml of freshly distilled hydroiodic acid ($d=1.56$) were placed in the flask B and then frozen in liquid nitrogen. Methyl-¹⁴C alcohol (100 μ l, 20mCi) was introduced into the reaction flask by vacuum distillation. The mixture was melted and heated at 80–85°C for one hour, then the product, methyl-¹⁴C iodide, was distilled through the purifying train into the product receiver E chilled with dry ice-acetone mixture.

3-Methyl-4-methyl-¹⁴C anisole. In the reaction flask F, 2.48g (10 mmoles) of 3-methyl-4-iodoanisole was dissolved into 15ml of dry ether, and 8 ml of *n*-hexane solution of *n*-butyllithium (containing 8.64 mmoles of the organo-metallic reagent) was added to this solution, then the reaction mixture was refluxed for 5 min under stirring. The mixture in the flask F was frozen in liquid nitrogen then the whole vacuum system was evacuated to 0.1 μ . The dry ice-acetone bath cooling the receiver E was removed off, and methyl-¹⁴C iodide was distilled into the flask F. The vacuum was replaced by one atmosphere of dry nitrogen and the mixture was refluxed for

one hour under stirring. After cooling to room temperature, the mixture was added with 20ml of water then extracted with ether. The solvent for the most part was removed by vacuum evaporation and the residue was submitted to a preparative gas-chromatography with a DC 550 column. Pure 3-methyl-4-methyl- ^{14}C anisole having 15.4 mCi was obtained in 77% yield (from methyl- ^{14}C alcohol).

3-Methyl-4-methyl- ^{14}C phenol (II- ^{14}C , 3,4-xylenol- ^{14}C): The dimethylanisole- ^{14}C was hydrolyzed in conc. hydrobromic acid by reflux for 10 hrs. After cooling, the reaction mixture was extracted with benzene and the combined extract was re-extracted with 5% sodium hydroxide solution. The aqueous extracts were combined and acidified with hydrochloric acid to convert the phenolate to the phenol then extracted again with benzene. The benzene solution was dried on Drierite then treated with activated charcoal to remove coloured impurities, filtered, freeze-dried to yield 386 mg (12.7 mCi) of crystalline 3-methyl-4-methyl- ^{14}C phenol, melting at 62~63°C, in both chemical and radiochemical yield of 94% from the anisole- ^{14}C . The infrared spectrum of the product was in agreement with that of non-radioactive authentic sample and a silica gel H thin-layer chromatogram developed with benzene: ethyl acetate: formic acid (40:10:1) showed that the compound was free of chemical and radiochemical impurities (Fig.2).

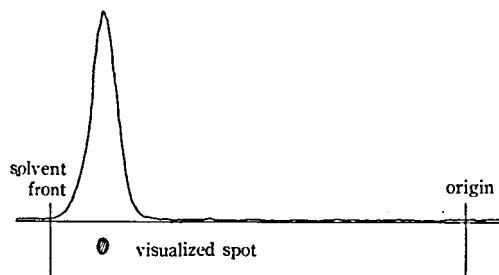


Fig.2. Radio-thin-layer chromatogram of 3,4-xylenol- ^{14}C .

T.L.C: adsorbent; silica gel HF, solvent; benzene-ethyl acetate-formic acid (40: 10: 1).

Radio-scanning: speed; 12.5 mm/min, time const; 3 sec, full scale; 300 Kcpm, slit width; 1 mm, with a windowless gas-flow counting system.

Visualization: with iodine.

3-Methyl-4-methyl- ^{14}C phenyl *N*-methylcarbamate (I- ^{14}C):

3-Methyl-4-methyl- ^{14}C phenol (300 mg, 9.9 mCi) was dissolved into dry isopropyl ether. To this solution 100 μl of dried and freshly distilled triethylamine and 1.04 g of methyl isocyanate were added. After stirring for 5 hrs at room temperature when no dimethylphenol- ^{14}C was recognized on the radio-thin-layer chromatogram, the solvent and excessively added methyl isocyanate were removed by vacuum evaporation. The residue, crude 3-methyl-4-methyl- ^{14}C phenyl *N*-methylcarbamate was recrystallized from petroleum ether. White crystalline carbamate- ^{14}C , melting at 78~78.5°C was obtained in 93% yield from the dimethylphenol- ^{14}C . Radiochemical and chemical purity was assayed by radio-chromatogram scanning and visualizing in silica gel H thin-layer, which gave one radioactive peak and visualized spot corresponding to an R_f -value of 0.75 of an authentic sample (Fig.3). The infrared spectrum also agreed with that of authentic sample.

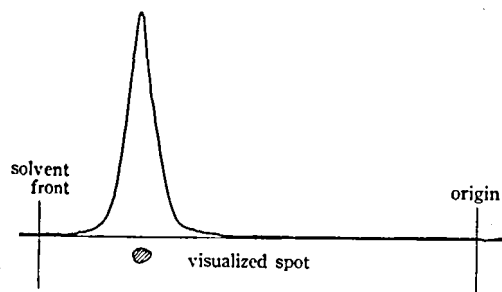


Fig.3. Radio-thin-layer chromatogram of 3,4-dimethylphenyl *N*-methylcarbamate.

T.L.C., Radio-scanning and visualisation were effected in the same manner described in Fig.2.

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Mating Vigour and Sexual Competitiveness of Chemosterilized Males of *Culex fatigans*.

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17. 不妊剤で処理された *Culex fatigans* 雄の交尾活性 On Prakash Raghuwanshi (Aligarh Muslim University, Aligarh, India) 44. 6. 19 受理

幼虫期に apholate で処理して不妊化した *Culex fatigans* の雄成虫と、正常の雄とをいろいろの割合で混在させ、雌と自由に交尾させて、不妊雄の交尾活性、交尾競争を卵のふ化で調べた。その結果不妊雄は交尾競争で劣ることはなかった。180匹の雄を不妊雄1:正常雄2の割合で、180匹の雌と籠の中で交尾させ、150卵塊を得た。この内57卵塊は不ふ化(ふ化率5%以下)であり、この期待値は50卵塊であった。

A number of workers have studied the effects of radiation and chemosterilization on the mating vigour and sexual competitiveness of male mosquitoes. Davis *et al* (1959) observed a significant deficiency in the mating vigour of irradiated males of *Anopheles quadrimaculatus*. Normal females when allowed to mate with irradiated and normal males in 1:1:1 ratios laid eggs having a hatch rate of 74.0% as compared to the normal hatch rate of 96.0%. Both the irradiated and chemosterilized males of *Musca domestica* were found to be equally competitive to normal males, but males of *A. quadrimaculatus* irradiated at 12,000 r or fed on 1.0% apholate suffered a reduction of about 26% (Schmidt *et al.* 1964). The same workers observed that irradiation of males at 10,000 r or exposure to 7 mg/ft² tepa residues for two hours had practically no effect on their mating behaviour. LaBrecque *et al.* (1962) observed that male houseflies got sterilized when fed on 1.0% apholate and were equally or even more aggressive than the normal males in their mating power. On the other hand a loss in the mating vigour of the males of *Aedes aegypti* when treated with chemosterilants has been observed by Dame *et al.* (1964).

The findings reported above suggest that the

effects of sterilizing agents on the mating vigour of males are rather specific and vary with the mode of treatment. As no attempt has been made to study the effects of apholate on the mating vigour and sexual competitiveness of *C. fatigans*, the phenomenon was studied by making treatments in the larval stage.

Materials and Methods

Test insect and chemical

The mosquitoes used in the present studies were drawn from the normal laboratory strain initially developed from larvae collected from a tank in Aligarh. The larval food consisted of dried yeast and blood albumen and the adults were reared on 10% glucose solution. The females were also fed on chicken blood.

Apholate was obtained through the courtesy of Dr. C. N. Smith of United States Department of Agriculture.

Experimental procedure

Five-day old larvae were released in water treated with 30 ppm apholate and were allowed to pupate. The pupae were sexed and the ones destined to become males were placed for emergence in wire frame cages covered over with muslin cloth and mosquito netting. The emerging males, when three-day old were released in cages