Journal of Labelled Compounds — July-September — Vol. V, No. 3

Synthesis of Farnesylacetic Acid-^{14}C and its Geraniol Ester (Gefarnate-^{14}C)

Masaaki HAZUE, Kunio MIYAKE and Michio ENDO

Pharmaceuticals Division Sumitomo Chemical Co., Ltd. Takarazuka, Hyogo, Japan.

Received on 24th February 1969

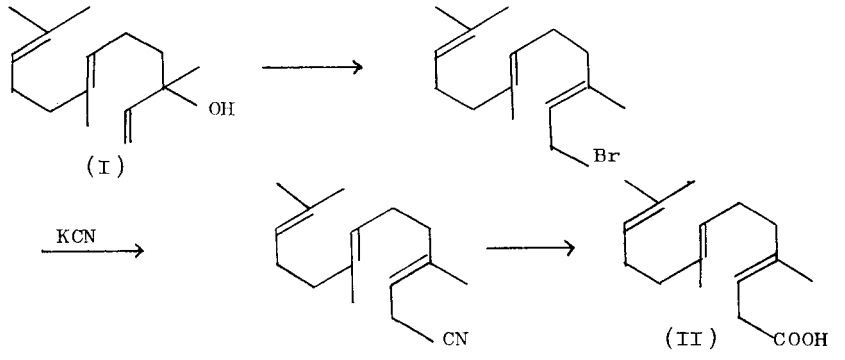
SUMMARY

Farnesylacetic acid and its geraniol ester (gefarnate) labelled with ^{14}C at the carbon-chain were prepared via *methyl cyclopropyl ketone-^{14}C. The intermediate, ketone-^{14}C was obtained in 63 % radiochemical yield from barium carbonate-^{14}C and from this ketone-^{14}C, farnesylacetic acid-^{14}C and gefarnate-^{14}C having a specific radioactivity of 1.0 mCi/mmole were prepared in 48.4 % and 37.2 % yield, respectively.*

INTRODUCTION

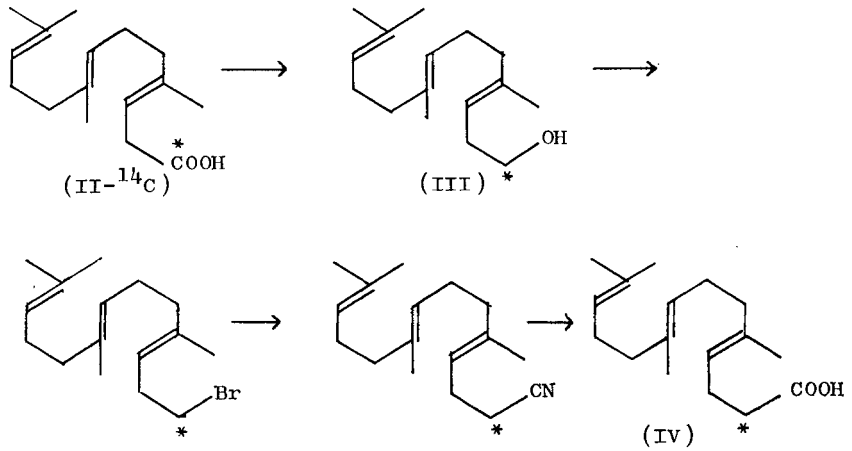
Studies in this laboratory on the metabolic fate of gefarnate required the preparation of farnesylacetic acid and gefarnate, the geraniol ester of the acid, labelled with ^{14}C at a site other than the carboxylic position. The present investigation was undertaken to see if a practical method could be worked out for preparing farnesylacetic acid, i.e., 5,9,13-trimethyl-4,8,12-tetradecatrienoic acid, and its geraniol ester, and much generally, the terpenoids labelled with ^{14}C at their carbon chain.

There are several possible modes of preparation for the carbon-chain labelled farnesylacetic acid. Lucius obtained homofarnesenic acid (II) starting with nerolidol (I) according to the following reaction scheme [1] :



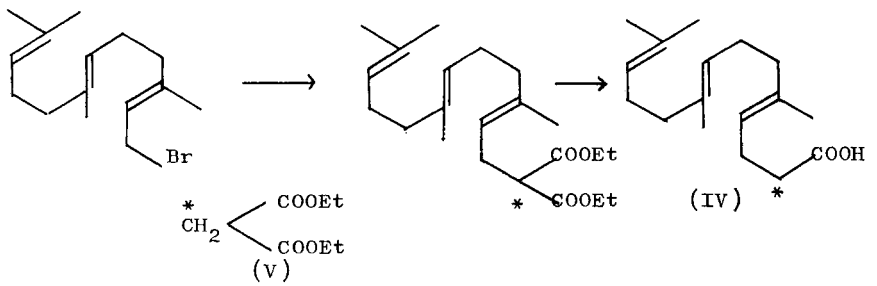
Taking the place of potassium cyanide by a radioactive one, homofarnesenic acid-1-^{14}C (II-^{14}C) which then can be reduced to homofarnesyl alcohol-^{14}C, may be prepared.

From the alcohol, farnesylacetic acid labelled at the 2-position (IV) may be possible to prepare through a reaction scheme similar to that described above :



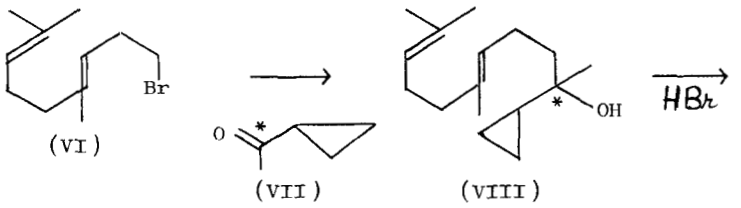
An alternative synthetic route for farnesylacetic acid reported by Dietrich and Lederer [3] consists of condensation of farnesyl bromide with diethyl malonate followed by saponification and decarboxylation of the resulting dicarboxylate.

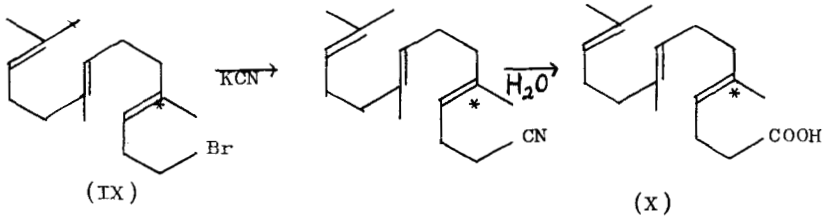
When diethyl malonate labelled with ^{14}C at the methylene position (V) is used, the intended labelled compound may be obtained :



More recently, Julia and his coworkers reported another synthetic pathway to yield farnesylacetic acid starting with methyl cyclopropyl ketone [4]. According to their procedure, the condensation of methyl cyclopropyl ketone labelled with ^{14}C at the carbonyl position (VII) with homogeranyl bromide (VI) followed by ring opening and dehydration of resulting cyclopropyl carbinol

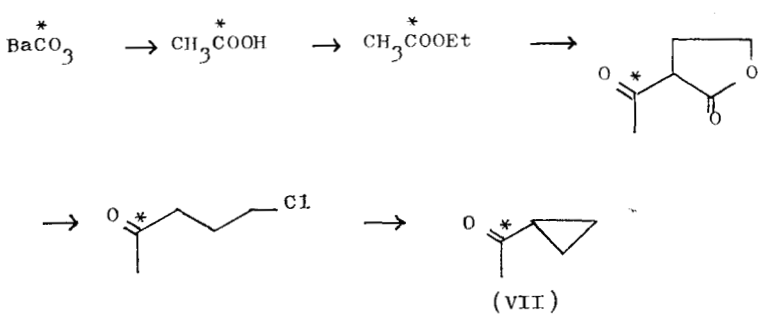
(VIII) may yield homofarnesyl bromide-4-^{14}C (IX). This labelled bromide then can be converted into the corresponding nitrile, and this is hydrolyzed to yield the intended farnesylacetic acid labelled at the 5-position (X) :





These three synthetic pathways were compared in respect of their over-all yield from the isotopic starting material, barium carbonate-^{14}C, and the number of reaction steps required. Consequently, it was concluded that it might be most appropriate to go through methyl cyclopropyl ketone-^{14}C, since this procedure requires 8 steps in all whereas the route passing through diethyl malonate-^{14}C requires 10 steps. The procedure based on Lucius was considered not to be appropriate owing to its low yield and complexity of the reaction sequence.

Thus the intermediate, methyl cyclopropyl ketone-^{14}C, was synthesized through the following reaction sequence :



And from the labelled ketone, farnesylacetic acid-5-^{14}C was prepared according to the direction of Julia and his coworkers with some modifications that come from a radiocative and a small scale synthesis. Preparative and purifying method of the labelled geraniol ester was also investigated.

DISCUSSION.

Preparation of methyl cyclopropyl ketone-(carbonyl)-^{14}C.

The general processes reported by Ohta [5] (from ethyl acetate to α-aceto-γ-butyrolactone) and reported by Cannon and his coworkers [6] (from the lactone to methyl cyclopropyl ketone) were followed for the radioactive and the small scale syntheses with some changes.

Thus, γ-butyrolactone was acetylated with ethyl acetate-^{14}C prepared from barium carbonate-^{14}C following a method given in "Organic Syntheses with Isotopes" [7]. Radioactive ethyl acetate, the amount of which was corresponding to about 80 % of stoichiometrically required, was added first to the reaction mixture containing γ-butyrolactone, and somewhat later the non-radioactive ester was added to make the total of ethyl acetate exceed the amount theoretically required. With this manner, the radiochemical yield of the crude product agreed with the chemical yield. When less amount of the ester-^{14}C than equivalent to the lactone was employed, it brought about difficulty in eliminating the excess γ-butyrolactone after the reaction. And in the case where just equivalent or excess amount of ethyl acetate-^{14}C was added at once, the radiochemical yield was lowered.

Inspite of Ohta's direction in which the reaction mixture was heated at 110-120° C after removal of the solvent, in the small scale synthesis, the reaction was completed by heating the mixture with solvent under reflux (85-90° C), and this brought on a higher yield of aceto-butyrolactone-^{14}C.

Since a considerable mechanical loss occurs at the separation and purification stages, the lactone-^{14}C was used for the next steps without further purification. This is equally true of 5-chloro-2-pentanone-^{14}C, so the radioactive haloketone was subjected to the next cyclization reaction without any purifying treatment.

Preparation and purification of farnesylacetic acid-^{14}C (X).

From methyl cyclopropyl ketone-^{14}C homofarnesyl bromide-^{14}C (IX) was comparatively readily obtained in the manner described by Julia and his coworkers. This bromide was treated with a large excess of potassium cyanide, and the resulting nitrile was hydrolyzed to the intended farnesylacetic acid-5-^{14}C (X).

On scanning of the radio-thin-layer chromatogram of this farnesylacetic acid-^{14}C, some radioactive impurities were found on the solvent front even after fractional distillation. (Fig. 1). To remove this persistent impurity, treat-

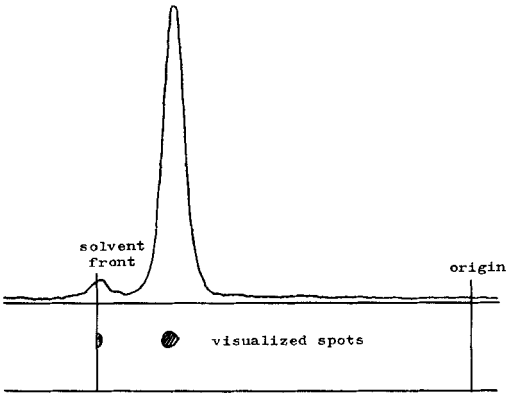


FIG. 1. Radio-thin-layer chromatogram of farnesylacetic acid-^{14}C (before column-chromatographic purification).

T. L. C. : adsorbent; silica gel HF, solvent; benzene-methanol-acetic acid (95 : 5 : 3)
Radio-scanning : speed; 12.5 mm/min, time const.; 3 sec, slit-width; 1 mm with a windowless gas-flow counting system.
Visualization : with anisaldehyde-sulphuric acid.

ment with a silica gel column has proved essential; without it, this impurity remained in the final product, gefarnate-^{14}C.

Esterification of farnesylacetic acid-^{14}C.

There are described many preparative methods for gefarnate starting with farnesylacetic acid [8]. A method, however, consisting with chlorination of farnesylacetic acid-^{14}C to the corresponding acid chloride with thionyl chloride followed by condensation with a large excess of geraniol under the presence of pyridine has proved to give the ester in the highest yield. The excess geraniol was removed by heating the mixture *in vacuo* after the reaction.

Chemical and radiochemical purity of gefarnate-^{14}C (X)

The infrared spectrum of gefarnate-^{14}C was essentially identical with that of an authentic sample. Scanning the thinlayer chromatogram, however, showed two radioactivity peaks of Rf 0.0 and 0.8 (Fig. 2). The main zone of Rf 0.8, which was superimposed on the iodine-visualized authentic spot, was scraped and gathered, and the compound adsorbed on the silica gel was extracted with ether and then re-chromatographed with the same thin-layer and solvent system. Contrarily to expectation, this treatment produced little, if any, effect upon the elimination of the impurity, i.e., the spot existing on the origin occured again but to a slightly lesser extent. In view of these facts, it seemed reasonable to assume that the radioactive impurity detected on the origin may have resulted from the decomposition of gefarnate-^{14}C during

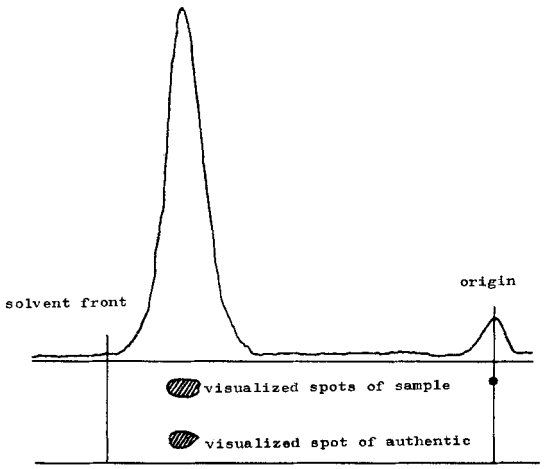


FIG. 2. Radio-thin-layer chromatogram of gefarnate-^{14}C
T. L. C. : adsorbent; silica gel HF, solvent; benzene-*n*-hexane (6 : 5).
Radio-scanning : speed; 12.5 mm/min, time const.; 3 sec, with a windowless gas-flow counting system, slit-width 1 mm.

the extraction and concentration processes or development on the silica gel layer.

Application of the method.

When a precursory bromine derivative is applicable, the method reported here would be equally suitable for preparation of the other 1-bromo-terpenoids labelled at the 4-position, so that 1-bromo-4-methyl-3-pentene-4-^{14}C would be obtained from methyl bromide, 1-bromo-4,8-dimethyl-3,7-nonadiene-4-^{14}C from 1-bromo-4-methyl-3-pentene, and so on. And these labelled 1-bromo-terpenoids, as occasion demands, may be converted into corresponding alcohols, nitriles and carboxylic acids etc.

EXPERIMENTAL.

For measurement of radioactivity of products, a liquid scintillation counting system (Kobe Kogyo, model GSL-163) was used, dissolving an aliquot of products into a liquid scintillator of the usual toluene-POPOP-p-terphenyl system. Radio-thin-layer chromatograms were scanned by an "Aloka Thin-Layer Chromatography Scanner, model TLC-28" under a scanning speed of 12.5 mm/min with a slit width of 1 mm.

Ethyl acetate-(carboxyl)-^{14}C.

A mixture of 2.19 g (25.67 mmoles, 58.0 mCi) of anhydrous sodium acetate-^{14}C prepared according to the direction of Murray [10] and dried for 5 hrs at 150° C under vacuum (10^{-4}-mmHg), and 13 ml of freshly distilled

triethyl phosphate was heated at 190-200° C under reflux for 3 hrs with moderate stirring. The reaction mixture was cooled, and the upper end of the condenser was attached to a vacuum manifold through a trap cooled at -20 °C. The mixture was distilled at 70° C under a vacuum of 10^{-4} mmHg with a small amount of triethyl phosphate being collected in the first trap and the product in a trap cooled with liquid nitrogen : yield; 1.49 g, 39.8 mCi. One milliliter of non-radioactive ethyl acetate was added to the reaction flask and again heated at 190-200° C for 1 hr; then the product was collected in the same way mentioned above : yield; 1.19 g, 12.4 mCi. By repeating this procedure once again, the radioactive ethyl acetate was almost completely collected into the receiver. The over-all radiochemical yield was 98.2 % (57.0 mCi) and specific radioactivity of the product was 1.36 mCi/mmole.

5-chloro-2-pentanone-(carbonyl)-^{14}C.

To γ-butyrolactone (4.3 g, 50 mmoles) dissolved in 5 ml of benzene was added 1.27 g (55 mmoles) of sodium. The reaction mixture was heated at 50-60° C then 3.69 g (41.9 mmoles, 57.0 mCi) of ethyl acetate-^{14}C was added dropwise during 30 min under stirring. The temperature of the mixture was gradually elevated to 85-90° C, and at this temperature the mixture was stirred for 5 hrs. Then 1.32 g (15 mmoles) of nonradioactive ethyl acetate was added dropwise, and refluxing was continued for 12 hrs. After cooling to room temperature, the mixture, without purification, was acidified by adding 10 ml of water then 17 ml of concentrated hydrochloric acid, cautiously. The mixture was distilled until no organic substances were observed in the distillate. The distillate was extracted with ether and dried with calcium chloride. The solvent and the excess ethyl acetate were removed by distillation through a 30-cm columm packed with glass helices. The residual crude 5-chloro-2-pentanone-(carbonyl)-^{14}C weighed 6.1 g, and the recovery of radioactivity was almost quantitative.

Methyl cyclopropyl ketone-(carbonyl)-^{14}C (VII).

In a flask fitted with a reflux condenser and magnetic stirrer were placed 4.3 g of sodium hydroxide pellets and 4.3 ml of water. To the solution, over a period of 20 min, 6.1 g of crude 5-chloro-2-pentanone-^{14}C was added. Boiling was initiated by slight heating and continued for 1 hr; then 7.5 ml of water was added slowly to the mixture over a 20 min period, and the mixture was heated under reflux for an additional hour. The condenser was arranged for distillation, and water-ketone mixture was distilled until all the organic layer was removed from the reaction mixture. The aqueous layer of the distillate was saturated with potassium carbonate and extracted with three 20 ml portions of ether. The extracts were combined and dried over calcium chloride for 3 hrs. The yield of methyl cyclopropyl ketone-^{14}C, b.p. 110-112° C (literature value, also 110-112° C [6]), n_D^{25}; 1,4218 (literature value, 1,4226 at 25° C [6]) was

2.92 g (34.7 mmoles) as chemical and 38.2 mCi (67.0 % from ethyl acetate -^{14}C) as radiochemical. The specific radioactivity was lowered to 1.1 mCi/mmole by dilution with non-radioactive ethyl acetate.

6,10-dimethyl-2-cyclopropyl-5,9-undecadiene-2-ol-2-^{14}C.

Methyl cyclopropyl ketone-^{14}C (2.9 g, 38.0 mCi) dissolved in 7.5 ml of dried ether was added dropwise to an ethereal solution of Grignard reagent* (36 mmoles) prepared from 1-bromo-4,8-dimethyl-3,7-nonadiene in the manner described by Julia *et al.* [4], at a temperature of 0-5° C. The stirring was continued at this temperature for another 3 hrs. Then the reaction mixture was allowed to stand overnight at ambient temperature. The mixture was hydrolyzed with saturated aqueous ammonium chloride and extracted with ether. The extract was dried on Drierite; then the solvent was evaporated *in vacuo* to yield crude cyclopropyl carbinol-^{14}C. Apparent yield was calculated as 90.5 % as chemical and 86.9 % as radiochemical. It was found, however, on the radio-thin-layer chromatogram, that this crude product included impurities such as decomposition product of the excess Grignard reagent and unreacted labelled ketone etc. and lower refractive index; n_D^{23} 1.4790 (literature value for a fractionnally distilled sample, 1.4822 at 21° C) was observed.

1-Bromo-4, 8, 12-trimethyl-3, 7, 11-tridecatriene-4-^{14}C (homofarnesyl bromide-4-^{14}C, IX).

To the above-mentioned crude carbinol-^{14}C, 10 ml of 47 % conc. hydrobromic acid was added under violent stirring at room temperature. The stirring was continued for 45 min. The mixture was extracted with petroleum ether, and then the combined extract was washed with saturated sodium chloride solution, sodium bicarbonate solution and again with saturated sodium chloride solution, successively. After drying over Drierite, the solvent was removed by distillation under vacuum. Crude homofarnesyl bromide-^{14}C, as residue, was obtained in both chemical and radiochemical yield of 85 % from the carbinol-^{14}C.

5, 9, 13-Trimethyl-4, 8, 12-tetradecatrienoic acid-5-^{14}C (farnesyl acetic acid-5-^{14}C, X).

The bromide-^{14}C obtained above was dissolved into 14.2 ml of N,N-dimethylformamide. To the solution, a saturated aqueous solution of 2.0 g of potassium cyanide was added, and the reaction mixture was heated at 70° C for 24 hrs under stirring. After adding of water, the mixture was extracted with benzene, washed with water and dried on Drierite; then the solvent was

* The concentration of the Grignard reagent was determined by titration.

evaporated *in vacuo*. The residual nitrile-^{14}C was hydrolyzed with an alcoholic solution of potassium hydroxide (20 g of potassium hydroxide, 100 ml of ethanol and 40 ml of water) by heating the mixture under reflux for 15 hrs. The alcohol for the most part was distilled off, then 65 ml of water was added, and about 55 ml of water was distilled along with a small quantity of oily organic substances. The residue was then extracted with petroleum ether to remove impurities in a Soxhlet-type liquid extractor. The aqueous layer was acidified with hydrochloric acid; then resulting crude farnesylacetic acid-^{14}C was extracted with petroleum ether, washed with water and dried on Drierite. Solvent was evaporated under vacuum, residue was fractionated *in vacuo* (0.2 mmHg), and 5.15 g (73 % from the bromide-^{14}C) of main fraction distilling at 155-160° C was collected. The radiochemical yield of the acid-^{14}C (19.4 mCi) was 71.5 % from the crude bromide-^{14}C and 52.6 % from methyl cyclopropyl ketone-^{14}C.

To remove a small quantity of radiochemical impurities, which was noticed on the solvent front of the radio-thin-layer chromatogram (Fig. 1) even after the fractional distillation, distilled acid-^{14}C was chromatographed over a silica gel H columm. Thus the acid-^{14}C separated from the impurities with a solvent system of benzene-methanol-acetic acid (95 : 5 : 3) was obtained in a yield of 92 %.

Radio-thin-layer chromatogram of this purified acid-^{14}C showed that virtually all of the radioactivity was present on the spot corresponding to the acid (Fig. 3). Refractive index, n_D^{26} 1.4832, agreed with the reported value, n_D^{26} 1.4830[4].

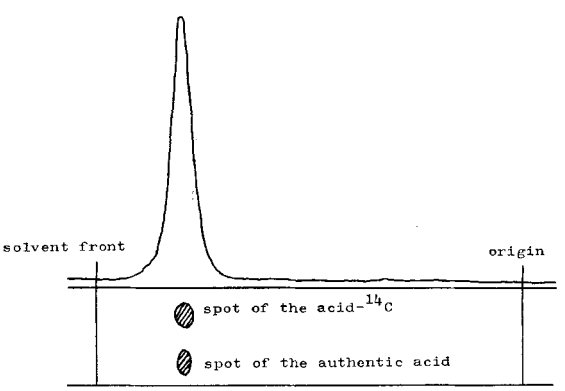


FIG. 3. Radio-thin-layer chromatogram of farnesylacetic acid-^{14}C (after column-chromatographic purification)

T. L. C.; radio-scanning and visualization were effected in the same manner described in Fig. 1.

5, 9, 13-trimethyl-4, 8, 12-tetradecatrienoyl chloride-5-^{14}C (farnesylacetyl chloride-5-^{14}C).

Farnesylacetic acid-^{14}C (2.58 g, 9.76 mCi) was dissolved in 15 ml of dried benzene. To this solution, 1.7 g of freshly distilled thionyl chloride was added dropwise at 0-5° C over a period of 10 min. The reaction mixture was stirred at this temperature for 2 hrs and allowed to stand overnight at room temperature. After removal of benzene and excess thionyl chloride, the residue was distilled at 0.2 mmHg, and the fraction that distilled at 135-140° C was collected to obtain 2.38 g (86 % from the acid-^{14}C) of the acid chloride-^{14}C.

Geranyl farnesylacetate-5-^{14}C (gefarnate-^{14}C).

The acid chloride-^{14}C was dissolved in 10 ml of toluene and cooled with an ice-water mixture. To this solution, geraniol dissolved in 10 ml of toluene with 0.81 g pyridine and cooled was slowly added dropwise during an hour. The reaction mixture was stirred at the chilled condition for another hour and then allowed to stand overnight at room temperature to complete the reaction. The mixture was filtered to remove crystalline pyridine hydrochloride; then the crystals were rinsed with ether. Filtrate and rinsing were combined and the solvent and the excess pyridine were evaporated *in vacuo*. The residue was heated at 90° C at 0.1 mmHg under a stream of nitrogen for 3 hrs until no geraniol was detected on a thin-layer chromatogram. Gefarnate-^{14}C having a specific radioactivity of 1.0 mCi/mmole was obtained in both chemical and radiochemical yield of 79 % (3.13 g, 3.97 mCi) from farnesylacetic acid-^{14}C.

ACKNOWLEDGEMENT.

The authors wish to thank the Sumitomo Chemical Co., Ltd. for the permission to publish this paper. They are also indebted to Messrs. T. Adach, Y. Makari and K. Kawahara for skilled technical help during this work.

REFERENCES

1. Lucius, G. — *Chem. Ber.*, **93** : 2663 (1950).
2. Hill, B. E., Lythgoe, B., Mirvish, S. and Trippett S. — *J. Chem. Soc.* **1955** : 1770. Jacobson, M. — *J. Am. Chem. Soc.*, **77** : 2461 (1955).
3. Dietrich, P. and Lederer, E. — *Helv. Chim. Acta.*, **35** : 1148 (1952).
4. Julia, M., Julia, S. and Guegan, R. — *Bull. Soc. Chim. France* **1960** : 1072.
5. Ohta, M. — Japan Patent 8271 (1956), *Chem. Abstr.*, **52** : 11904 (1958).
6. *Org. Syntheses*, **31** : 74 (1951).
7. "Organic Syntheses with Isotopes" part I, p. 416 (Editors, A. Murray, III and D. L. Williams) Interscience Publishers, Inc., New York (1958).
8. Cardani, C., Cavalleri, P. and Adami, E. — *J. Medicinal Chemistry*, **6** : 457 (1963).
9. Ropp, G. A. — *J. Am. Chem. Soc.*, **72** : 2299 (1950).
10. "Organic Syntheses with Isotopes" part I, p. 34 (Editors, A. Murray, III and D. L. Williams) Interscience Publishers, Inc. New York (1958).