Synthesis of Geranylgeraniol-2-^{14}C

All *trans* geranylgeraniol, III (3, 7, 11, 15-tetramethyl-2, 6, 10, 14-hexadecatetraene-1-01) has been implicated as a precursor in biosynthesis of carotenoid pigments in a number of systems as the pyrophosphate ester [1]. We became interested in using the ^{14}C labelled alcohol to study biosynthesis in the intact tomato fruit and tomato plastids. Consequently it was necessary to devise a method of synthesis for this alcohol. The synthesis described herein provides a relatively simple scheme to obtain isoprenoid alcohols of high specific radioactivity.

Several methods have been used for the synthesis of isoprenoid alcohols [2, 3, 4]. These methods are not entirely suitable for the synthesis of ^{14}C isoprenoid alcohols either, because the label must be introduced relatively early in the procedure [2, 3] or the sterochemical course of the reaction sequence is not favorable [4].

Recently isoprenoid alcohols have been prepared [5, 6] by using a modified Wittig type reaction [7]. Although this procedure is especially suited to synthesis of ^{14}C labelled isoprenoid alcohols, it has not been used previously. Using it, the label can be introduced near the end of the reaction sequence, and a high *trans-cis* ratio can be obtained.

Thiophene free reagent grade benzene was dried over calcium hydride and passed through a silica gel column before use. Ethyleneglycol dimethyl ether was distilled from calcium hydride before us. Triethyl phosphite, ethyl acetate and ethyl acetoacetate were purified by fractional distillation. Ethyl bromoacetate, nerolidol (Aldrich *), phosphorus tribromide (practical), lithium aluminum hydride and sodium hydride (58.9% in oil) were used without further purification. Methyl bromoacetate-2-^{14}C (Nuclear Chicago) had a specific activity of 4.44 mC/mM.

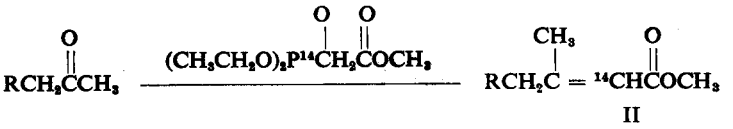
A sample of all-*trans* geranyllinalool (Hoffman La Roche and Co., Basel, Switzerland) was converted to III by bromination of the alcohol and hydrolysis of the rearranged bromide [2].

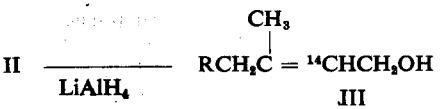
* Use of trade names of specific material does not constitute a recommendation by the U. S. Department of Agriculture to the exclusion of others which may also be available.

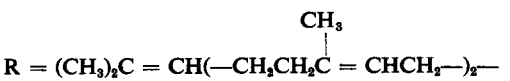
Thin layer chromatography was performed on silica gel G using benzene-ethyl acetate (95 : 5) as the mobile phase. Visualization was by iodine vapors. Column chromatography was carried out on a 2.5 × 70 cm column packed with 20 % silver nitrate on silicic acid-Super cel (2 : 1).

Gas chromatography was performed on an Aerograph 600-D equipped with a flame ionization detector. A column 1/8 in. in diameter and 5 ft. in length packed with 5 % diethylene glycol succinate polyester on Chromosorb W was used. Column temperature was 185° C with a nitrogen gas flow rate of 28 ml/min.

The procedure used to synthesize III-2-^{14}C is as follows :







All-*trans* farnesylacetone (I) [2] was reacted with methyl diethyl phosphonoacetate-2-^{14}C to give the ester II. Formation of the double bond proceeded with a 70 : 30 ratio of *trans* to *cis* isomers being obtained. Chromatography of the ester on a silicic acid-silver nitrate column [8] yielded material having a 95 : 5 *trans-cis* ratio. Reduction with lithium aluminum hydride gave ^{14}C labelled III.

6, 10, 14-trimethyl-5, 9, 13-pentadecatriene-2-one (*I*). The all-*trans* isomer was prepared as previously described [3].

Methyl Diethyl Phosphonoacetate-2-^{14}C. Triethyl phosphite, 2.01 g (12 mM) was mixed with 0.5 mC methyl bromoacetate-2-^{14}C (17.1 mg, 0.1 mM). To this was added 2.055 g (12 mM) of ethyl bromoacetate as a carrier. The mixture was heated 4 hr at 150° C and finally for 10 min at 200° C. Nitrogen gas was passed in during the entire reaction period to remove the ethyl bromide. A 2.5 g quantity (93 % yield) of the phosphonoacetate was isolated.

Geranylgeraniol-2-^{14}C (*III*). To 15 ml of 1,2 dimethoxy ethane was added 0.305 g (7.61 mM) of sodium hydride. A solution containing 1.7 g (7.6 mM) of methyl diethyl phosphonoacetate-2-^{14}C in 10 ml of 1,2 dimethoxyethane was added to the sodium hydride slurry. The temperature was maintained at 18° C during the addition. The solution was warmed to room temperature

and stirred for 45 min. To the yellow suspension a solution of 2.0 g of all-*trans* I in 15 ml of 1,2 dimethoxyethane was added over a 30 min period. The reaction mixture was stirred at 60° C for 2.5 hr and then allowed to stir overnight at room temperature. After dilution with 300 ml water, extraction with ether and removal of the dried ether, 1.9 g (73.8% yield) of II was recovered. Gas liquid phase chromatography (g.l.p.c.) showed a 70 : 30 *trans-cis* ratio.

The crude reaction mixture was chromatographed on a silicic acid-Super Cel (2 : 1) column containing 20% silver nitrate. The all-*trans* isomer was partially separated from the mono-*cis* isomer with 95 : 5 benzene-ethyl acetate as solvent. Several passes of the ester were required for adequate separation. Gas chromatography of the purified ester showed 95% *trans* isomer. An infrared spectrum gave bands at 1,718 cm^{-1} and 1,650 cm^{-1} indicating an α, β unsaturated ester. Thin layer chromatography showed that virtually all of the radioactivity was present in the spots corresponding to the ester.

A solution containing 0.458 g (1.38 mM) of II was reduced with 1.04 g (2.7 mM) of $LiAlH_4$ at −15° C for 1.5 hr [4]. A total of 0.365 g (78% yield) of III was isolated.

Infrared spectrum, g.l.p.c. and t.l.c. indicated that approximately 12% of the ester remained unreduced. A small scale experiment indicated that the alcohol can be separated from the ester by the t.l.c. system described above. The crude reaction mixture was used directly in the preparation of the pyrophosphate [4].

Infrared and n.m.r. spectra were practically identical with spectra of III isolated from linseed oil [9] and authentic unlabelled III. The R_f on t.l.c. and R on g.l.p.c. were identical to that of authentic unlabelled III. The specific activity was 0.203 mC/mM.

ACKNOWLEDGEMENTS.

The author is grateful to Dr. Marion L. Miles of N.C. State University for help in obtaining and interpreting the n.m.r. and infrared spectra. In addition, appreciation is expressed to Hoffman La Roche, Basel, Switzerland for the sample of geranyllinalool.

William M. WALTER, Jr.

Department of Food Science, North Carolina State University,
Raleigh, North Carolina,
and Southern Utilization Research and Development Division,
Agricultural Research Service, USA

REFERENCES

1. GOODWIN, T. W. — In Chemistry and Biochemistry of Plant Pigments, T.W. Goodwin, Ed., Academic Press, New York, 143-169 (1965).

2. ISLER, O., RUEGG, R., CHOPARD-dit-JEAN, L. H., WINTERSTEIN, A. and WIS, O. — *Helv. Chim. Acta*, **41** : 786 (1958).
3. NAZAROV, I. N., GUSEV, B. P. and GUNAR, V. I. — *Zhur. Obshchei. Khim.* **28** : 1198 (1958).
4. POPJAK, G., CORNFORTH, J. W., CORNFORTH, Rita H., RYHAGE, R. and GOODMAN, D. S. — *J. Biol. Chem.*, **237** : 56 (1962).
5. JACKMAN, L. M., RUEGG, R., RYSER, G., PLANTA, C. V., GLOOR, U., MAYER, H., SCHUDEL, O., KOFLER, M. and ISLER, O. — *Helv. Chim. Acta*, **48** : 1332 (1965).
6. AZEROD, R. and CYROT, M. O. — *Bull. Soc. Chim. France*, **9** : 3740 (1965).
7. WADSWORTH, W. S., Jr. and EMMONS, W. D. — *J. Am. Chem. Soc.*, **83** : 1733 (1961).
8. JAMES, A. T. — In New Biochemical Separations, JAMES, A. T. and MORRIS, L. J. Ed., D. Van Nostrand Company, Ltd. London, England, 310 (1964).
9. FEDELI, E. *et al.* — *J. Lipid Res.*, 7 : 437 (1966).