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Synthesis of Geranylgeraniol-2-¹⁴C

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 ¹⁴C 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 ¹⁴C isoprenoid alcohols either, because the label must be introduced relatively early in the procedure (^{2, 3}) or the stereochemical 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 ¹⁴C 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 use. 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-2-¹⁴C (Nuclear Chicago) had a specific activity of 4.44 mC/mM.

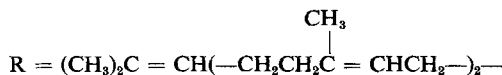
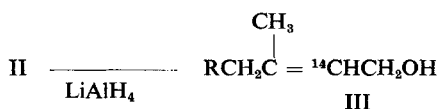
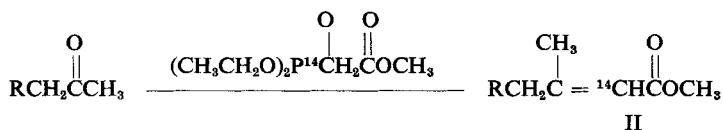
A sample of all-*trans* geranylinalool (Hoffman La Roche and Co., Basel, Switzerland) was converted to III by bromination of the alcohol and hydrolysis of the rearranged bromide (²).

* 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-2-¹⁴C is as follows :



All-*trans* farnesylacetone (I) ⁽²⁾ was reacted with methyl diethyl phosphonoacetate-2-¹⁴C 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 ¹⁴C 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-2-¹⁴C. Triethyl phosphite, 2.01 g (12 mM) was mixed with 0.5 mC methyl bromoacetate-2-¹⁴C (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-2-¹⁴C (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-2-¹⁴C 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.

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Some observations on the Labelling of P_2S_5 with carrier-free ^{32}P formed in elementary Sulphur by the Nuclear Reaction $^{32}S(n,p)^{32}P$

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P_2S_5 labelled with ^{32}P can be obtained by synthesis or isotopic exchange. On the labelling of P_2S_5 by isotopic exchange there are already some indications. Thus, J.E. Casida⁽¹⁾ obtained P_2S_5 labelled with ^{32}P by an isotopic exchange between $HNa_2^{32}PO_4$ or $H_3^{32}PO_4$ and P_2S_5 . M. Dubini and G. P. Perucca⁽²⁾ studied the labelling of P_2S_5 with the carrier-free ^{32}P present in the residu of distillation of the neutron irradiated elementary sulphur. The exchange yield after 3 hours at 300° C was 42%. If a mixture of red phosphorus and elementary sulphur (ratio 2 : 5) were reacted in the presence of the residual ^{32}P the labelling yield of P_2S_5 was 50-95%⁽²⁾. From these paper^(1, 2) we drew the conclusion that the labelling of P_2S_5 by isotopic exchange can be carried out with ^{32}P in various chemical forms. It is clear that the chemical form of the residual ^{32}P ⁽²⁾ is not similar with $HNa_2^{32}PO_4$ or $H_3^{32}PO_4$ ⁽¹⁾.

In our experiments we also studied the labelling of P_2S_5 with the carrier-free ^{32}P formed in elementary sulphur by the nuclear reaction $^{32}S(n, p)^{32}P$, but in different experimental conditions. The purpose of this paper was to obtain some information on the labelling mechanism of P_2S_5 . This experiments were put in accordance with our earlier observations⁽²⁾ on the chemical state of ^{32}P atoms in the elementary sulphur. Thus, in the deaerated sulphur targets ^{32}P atoms are stabilized in an elementary chemical state, but in the untreated sulphur targets in an oxydated chemical state. Also, we observed⁽⁴⁾ that the carrier-free ^{32}P formed in elementary sulphur can be adsorbed on the red phosphorus.

Taking into account these observations the labelling of P_2S_5 with the carrier-free ^{32}P in following experimental conditions was studied.

A — Synthesis of $^{32}P_2S_5$ from red phosphorus and irradiated elementary sulphur. (ratio 2 : 5).

B — Synthesis of $^{32}P_2S_5$ after the removal of sulphur target by the adsorbtion of ^{32}P on the red phosphorus and then its reaction with the elementary sulphur (unirradiated sulphur)

C — Isotopic exchange between ^{32}P atoms present in the sulphur targets and P_2S_5 .

In all cases the experiments were carried out with untreated and deaerated sulphur targets.