Synthesis of 3,3-dimethylallyl-1-^{14}C alcohol and 3,3-dimethylacrylic-1-^{14}C acid

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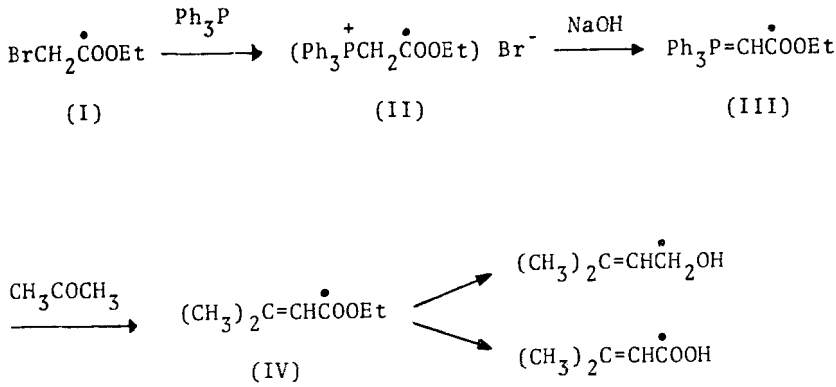
In connection with our work on the biosynthesis of isoprenic alkaloids [1] there arose the need of 3,3-dimethylallyl-1-^{14}C alcohol to be tested as a precursor in one of our experiments. Radioactive 3,3-dimethylallyl alcohol had been previously prepared but the label was localized at other carbon-atom than the required for our project (e.g. at the methyl groups) [2].

We wish to report that the title products were synthesized according to the following sequence (see Scheme) :



SCHEME. Labelled carbon-atoms are indicated with heavy dots.

Ethyl bromoacetate-1-^{14}C (I) reacted with triphenylphosphine to produce radioactive (carbethoxymethyl)triphenylphosphonium bromide (II) [3] which was decomposed with sodium hydroxide to the corresponding (carbethoxymethylene)triphenylphosphorane (III). The latter, upon reaction with acetone [4], afforded ethyl 3,3-dimethylacrylate-1-^{14}C (IV) which was purified by high-vacuum distillation. Compound IV, on reduction with lithium aluminium-hydride [2], yielded 3,3-dimethylallyl-1-^{14}C alcohol whose physical properties were identical to those from an authentic sample.

Alternatively, compound IV was saponified with sodium hydroxide yielding 3,3-dimethylacrylic-1-^{14}C acid with physical properties identical to those obtained from an authentic sample. This labelled compound had been previously synthesized but following a different approach [5].

Both final products showed on radio-thin-layer chromatography a radiochemical purity higher than 99 %.

EXPERIMENTAL.

Melting points were determined with a Fisher-Johns hot-plate and are uncorrected. I.r. spectra were measured with a Perkin-Elmer Infracord spectrophotometer. N.m.r. spectra were recorded with a Varian A-60 spectrometer. Radioactive samples were measured with a Packard Tri-Carb model 3305 liquid scintillation spectrometer in the usual scintillation solutions. T.l. chromatography was conducted on aluminium oxide (Merck, PF_{254}, type E) in chloroform : methanol mixtures. Sodium acetate-1-^{14}C was purchased from the Comisión Nacional de Energía Atómica, Argentina. Solvents were removed under diminished pressure below 50°.

Labelled (carbethoxymethyl)triphenylphosphonium bromide (II). — Ethyl bromoacetate-1-^{14}C (1.77 g, 3.0×10^8 dpm/mmole) [6] in benzene (3 ml) was slowly added onto a solution of triphenyl phosphine (5 g) in benzene (10 ml). The mixture was left overnight at 0°. The solid was filtered off and washed with benzene : pet. ether (1 : 1). Compound II (3.9 g) had m.p. 152-153° and spec. act. 3.0×10^8 dpm/mmole.

Labelled (carbethoxymethylene)triphenylphosphorane (III). — Compound II (3.8 g) was dissolved in water (80 ml), and the cooled solution was made basic with 0.5N NaOH. After 12 h at 0° the solid was collected by filtration. The dried product weighted 2.52 g and had m.p. 125-127°; spec. act. 2.9×10^8 dpm/mmole.

Ethyl 3,3-dimethylacrylate-1-^{14}C (IV). — Compound III (1.25 g) was dissolved in dry acetone (15 ml), and the solution was heated in a sealed tube at 100° for 24 h. The acetone in excess was then evaporated, and the solid residue was extracted with petroleum ether (60-80) (5×10 ml). The ethereal extract was evaporated and the residue was distilled at reduced pressure (75°, 40 Torr) yielding 98 mg of IV. I.r. and n.m.r. spectra were identical to the obtained from an authentic sample. Spec. act. 2.9×10^8 dpm/mmole.

3,3-Dimethylallyl-1-^{14}C alcohol. — Compound IV (75 mg) in dry ether (25 ml) was treated at 0° and under stirring with $LiAlH_4$ (30 mg) added in small portions. The excess of reagent was destroyed by addition of ethyl acetate. The mixture was treated with saturated $NaHCO_3$ solution (3.5 ml) and extracted with ether (5×10 ml). The residue obtained for evaporation of the dried ($MgSO_4$) extract weighted 50 mg. It was homogeneous on t.l.c. and identified as 3,3-dimethylallyl alcohol by comparison of its i.r. and n.m.r. spectra with an authentic sample. Spec. act. 3.0×10^8 dpm/mmole.

3,3-Dimethylacrylic-1-^{14}C acid. — Compound IV, obtained as above described, (98 mg) was treated with 5N NaOH (0.5 ml), and the mixture was heated at 80° under continous stirring until it became a homogeneous solution (about 3 h). It was then made acid with 6N HCl, and the solid thus

formed was filtered off. The dried product (47 mg, m.p. 68-70°), homogeneous on t.l.c., was identified as 3,3-dimethylacrylic acid by comparison (i.r., n.m.r.) with an authentic sample. Spec. act. 3.1×10^8 dpm/mmole.

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Preparation of ^{131}I-labelled elastase

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Elastase is a pancreatic proteolytic enzyme with the action to solubilize elastic fibres [1, 2]. For the studies relating to its intestinal absorption and metabolic fate, ^{131}I-labelled elastase was required.

Our first attempts to prepare tritiated elastase by the Wilzbach technique were unsuccessful, which is based upon Steinberg's method for the tritiation of lysozyme [3]; a large extent of decomposition of the compound was observed during the labelling process. Then Greenwood's [4] method was applied to prepare ^{131}I-labelled elastase. The present paper is concerned with the ^{131}I-labelling of elastase and the purity of the preparation.

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