PREPARATION OF TRITIUM-LABELLED JASMONIC ACID, ETHYL DIHYDROJASMONATE AND ITACONIC ACID

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SUMMARY

A method for tritium labelling of jasmonic acid, ethyl dihydrojasmonate, and itaconic acid involving metal catalyzed hydrogen isotope exchange is described. The procedure is a convenient one-step synthesis and specific activities in the range of 3 to 30 GBq/mmol were obtained.

INTRODUCTION

Jasmonic acid, ethyl dihydrojasmonate and itaconic acid are important plant growth regulators. For physiological experiments we needed these compounds tritium-labelled with specific activities in the range of 1 to 10 GBq/mmol. Until now no information has been published on the tritium labelling of jasmonic and itaconic acids.

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RESULTS AND DISCUSSION

Previous attempts to label itaconic acid with tritium by the Wilzbach technique [1] were unsuccessful due to the formation of many highly tritiated impurities making purification impossible. Using metal catalyzed hydrogen-tritium exchange developed by Evans et al. [2] we observed hydrogenation of itaconic acid. A substance without uv-activity was obtained. Therefore we modified this method by working without a tritium atmosphere during isotope exchange reaction.

In the present method palladium black [3] was pre-activated with tritium gas in a solvent in which the substance to be tritiated is soluble. After activation of the catalyst with tritium gas the substance was added. The mixture was stirred for 3-4 hours at room temperature and the catalyst separated by centrifugation. After that labile tritium was removed by repeated evaporations with ethanol in vacuo. For analysis of substances mentioned above we used chromatography on thin-layer plates. For comparison reference substance was located on the plate. Moreover the identity of substances was checked by biological experiments. Labelling positions in the labelled molecules were not investigated.

For labelling itaconic acid ethanol was used as solvent. Fig. 1 shows a radiogram of the substance after labelling. Rf-value of the main peak agreed with the Rf-value of itaconic acid. Purification from the impurity which appears at the start point of the radiogram was carried out very easily with a silica gel column. The specific activity of itaconic acid obtained was 3,1 GBq/mmol. The biological activity of the labelled product and the melting point were identical with itaconic acid.

Ethanol was also used as solvent for labelling of jasmonic acid. The radiogram in Fig. 2 shows that during the labelling process a radiochemically pure compound was formed. The Rf-value of the radioactive product and its biological activity were identical with jasmonic acid. In this case no purification was necessary. The specific activity achieved was 7,0 GBq/mmol.

Labelling of ethyl dihydrojasmonate by hydrogenation of jasmonic acid and followed esterification was unsuccessful. After hydrogenation of jasmonic acid there were many radioactive impurities in addition to dihydrojasmonic acid and purification of the crude product was difficult. Therefore we applied the method mentioned above. Diethyl ether was used as labelling solvent.
The activation of the catalyst with tritium gas was achieved at a temperature of -78°C in order to have a low vapour pressure of the diethyl ether. During the labelling procedure the mixture was stirred at room temperature. After labelling ether was removed by evaporation in vacuo. Rf-value of the radioactive peak shown in Fig. 3 agreed with ethyl dihydrojasmonate. The biological behaviour of the labelled product was the same as ethyl dihydrojasmonate. In this example also purification was not necessary and the specific activity was 34,1 GBq/mmol.
The specific activity of the ethyl dihydrojasmonate is higher than the activities achieved in the other two examples. This is probably due to ethanol (or other hydroxylic solvents) reducing the specific activity of the tritium gas adsorbed on the catalyst, by exchange on the surface of catalyst [3]. In contrast to other isotope exchange reactions this method is fast and the labelled compounds obtained are of very high radiochemical purity. The method may be suitable for certain compounds with multiple bonds or containing halogen atoms.

EXPERIMENTAL

Methods

For radio-t.l.c. Silufol plates UV 254 (Sklarny Kavalier CSSR) were used. Radiograms were obtained on a Berthold Scanner II. The activity measurements were obtained using a Beckman LS-233. The palladium black was produced as described by Loew [3] in our own laboratory.

Activation of palladium black with tritium gas

Palladium black (70mg) and 1-2ml of an inert solvent were stirred magnetically in a reaction vessel in the presence of tritium gas with a pressure of 400-500 mm Hg. When the pressure in the tritium apparatus [5] was constant, tritium was adsorbed on uranium.

Labelling of itaconic acid

Itaconic acid (180mg), ethanol (1,5ml) and the activated catalyst were stirred magnetically for 3 hours. Then the catalyst was removed by centrifugation and labile tritium was removed by repeated evaporations with ethanol in vacuo. Column chromatography with silica gel yielded 80mg of radiochemically pure itaconic acid with a specific activity of 3,1 GBq/mmol by elution with chloroform : ether (40 : 10). m.p. 163-165°C.

Labelling of sodium jasmonate

The sodium salt of jasmonic acid (110mg) were used for labelling following the procedure as described above. 98mg of labelled sodium jasmonate with a specific activity of 7,0 GBq/mmol was obtained. Purification was not necessary.

Labelling of ethyl dihydrojasmonate

Palladium black was activated in 2ml diethyl ether at a temperature of -78°C as described above. Ethyl dihydrojasmonate (250mg) was there added. The mixture was stirred magnetically for 4 hours. After separation of the catalyst, the ether was evaporated in vacuo. 233mg of radiochemically pure ethyl dihydrojasmonate with a specific activity of 34,1 GBq/mmol was obtained.

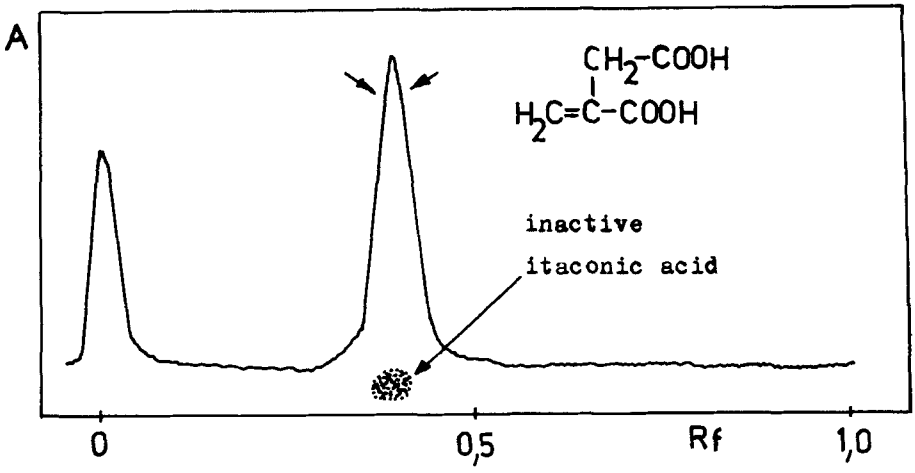

Fig. 1. Radiogram of itaconic acid directly isolated from the labelling reaction. Solvent system chloroform: ether: acetic acid (40:10:5).

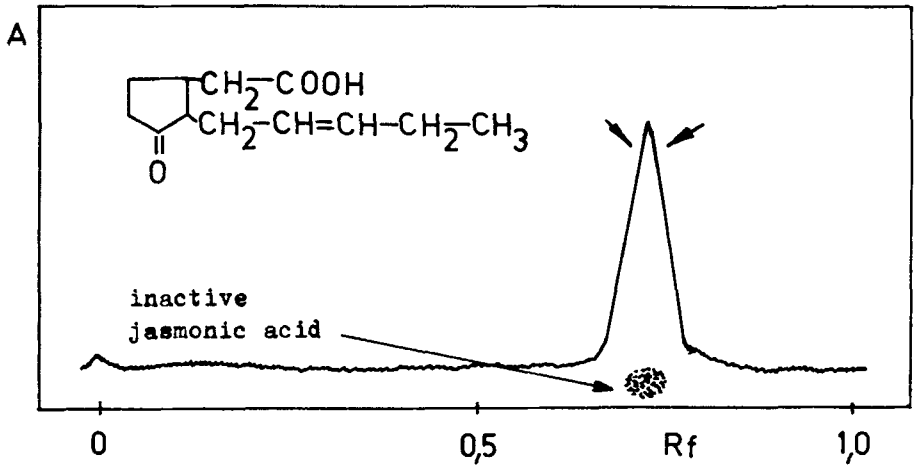

Fig. 2. Radiogram of jasmonic acid directly isolated from the labelling reaction. Solvent system benzene: ether: acetic acid (40:9:1).

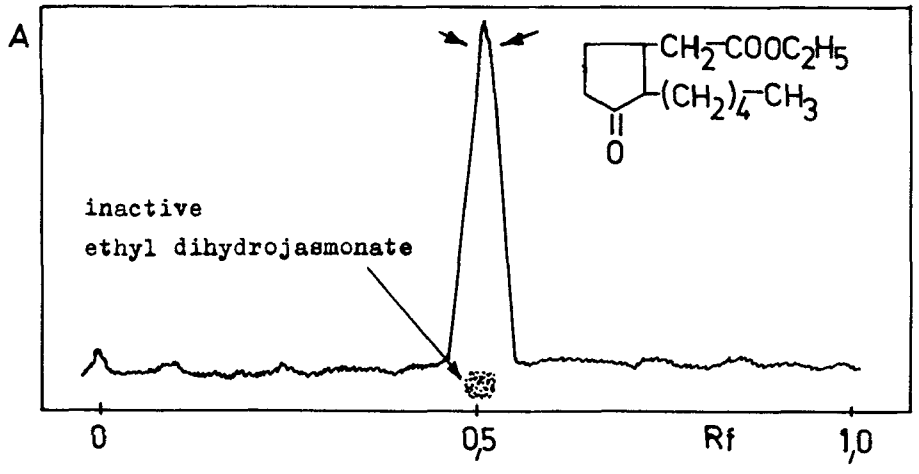

Fig. 3. Radiogram of ethyl dihydrojasmonate directly isolated from the labelling reaction. Solvent system benzene: ethanol (95:5).

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