

LABELLING OF BREFELDIN-A BY CATALYZED ISOTOPIC EXCHANGE WITH TRITIUM GAS.

Ph. MAETZ, F. SOBRIO, C. MIOSKOWSKI and B. ROUSSEAU*

Service des Molécules Marquées, Département de Biologie Cellulaire et Moléculaire,
C. E. A. Saclay, , F-91191 Gif sur Yvette.

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Summary

The hydrogen isotope exchange of Brefeldin-A with tritium gas (T_2) in 1,4-dioxane was studied over a commercial palladium catalyst supported on diatomaceous earth (5% metallic weight). The effect of the gas phase was studied. Addition of air in the gas phase led to an increase of the catalytic activity. Moreover, exchange was enhanced when the air/ T_2 ratio reached a value of 4. This latter result is interpreted, principally, in terms of a strongly poisoning of the metallic particles together with an effect of the T_2 partial pressure. Through the comparison with Pd(II) oxide, the role of the support is also shown. The specific activities ranged up to 2.8 Ci/mMoles. The labelled product was analyzed by 3H NMR. Possible mechanisms for tritium incorporation are discussed and two different adsorbed species, on the catalyst surface, are involved.

I. INTRODUCTION.

Drugs labelled with tritium or deuterium are widely used as research tools in the life science. Therefore, it is important to investigate whether one-step catalytic reactions can be developed to replace the time consuming and somewhat demanding synthetic procedures that are often necessary.

Catalyzed hydrogen isotope exchange reactions are widely used under both heterogeneous and homogeneous conditions. Often such reactions are employed to study mechanisms of catalysis rather than for the synthesis of labelled compounds.

However, the labelling of organic compounds with tritium, either by catalyzed exchange reaction or by the Wilzbach method, has been extensively studied (1). Hydrogen isotope exchange with tritium gas and/or tritiated water has been studied using heterogeneous and homogeneous catalysts of many group VIII metals. A wide variety of tritium labelled organic compounds, aromatics, aliphatics, purines, aromatic amines and amino acids, carbohydrates and

*To whom all correspondence should be addressed

steroids, with high specific activity and radiochemical purity, have already been obtained using principally platinum or palladium catalysts (2,3,4). Homogeneous rhodium trichloride was found to promote *ortho*-tritiation with high regioselectivity in a broad range of aromatic carboxylic acids, amides and aralkylamines (5). However, in the case of more complex molecules, only a few papers have been published by Myasoedov et al. (6,7).

From a biological point of view Brefeldin A (BFA) (fig. 1) presents a great interest (8). BFA has a profound effect on the Golgi apparatus and can dramatically alter the membrane traffic. These findings provided cell biologists with a potent tool for investigating fundamental questions about organelle identity and membrane traffic. Therefore, the synthesis of the tritium labelled molecule should be helpful for a better understanding of its biological action. Moreover, the structure of BFA is complex (see fig.1), double bonds, alcoholic and lactone functions, five asymmetric carbons, and therefore is representative of a wide range of molecules of biological or pharmaceutical interest. So, BFA seemed to be a good model to us for the development of a new method of labelling by isotopic exchange.

The aim of our work was to probe the possibility of using heterogeneous catalysts to obtain labelling BFA. In this paper, some results obtained in the heterogeneous exchange reaction between BFA and tritium gas on a palladium catalyst supported on diatomaceous earth are reported together with the analysis of labelled BFA by ^3H N.M.R.. The influence of the gas phase composition on the catalytic activity was studied. Possible exchange mechanisms are also discussed.

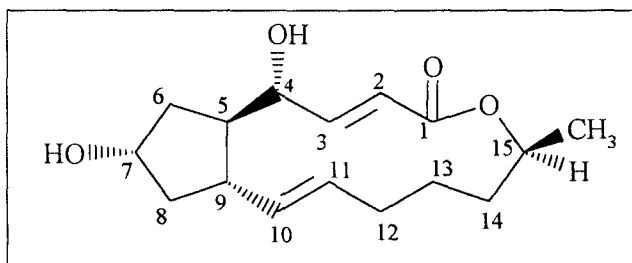


Figure 1 : BREFELDIN A

II RESULTS.

As indicated above, the gas phase composition, especially the air/ T_2 ratio, was varied, so the exchange reactions were performed with a variable quantity of tritium, 5 to 10 Ci. In Table 1 the results obtained on Pd supported on diatomaceous earth are reported. Both the yield determined by UV detection (215 nm) using standard solutions as reference and the radiochemical yield are indicated, as well as the total radioactivity amount obtained. In the first column, both gas phase composition and ratio are indicated, the pressure was always 0.25 atm. . Each experiment was reproduced at least two times.

When the gas phase contained only pure T_2 , the catalytic activity was the lowest we obtained. The yield in BFA was about 70%, but tritium incorporation is not high and led to a low specific activity of 0.1 Ci/mMole. The radiochemical analysis showed only three reaction products.

Air addition to the gas phase led to an increase in the catalytic activity. When air/T₂ ratio was increased from 0.3 to 2, the chemical yield in BFA strongly diminished, and the amount of labelled BFA dropped dramatically. In parallel, there was an increase in the radioactivity amount due to the formation of hydrogenated compounds, with a radiochemical yield of about 90%.

In the case of a higher air/T₂ ratio the behaviour of the catalyst changed again. At a ratio of 4, the total radioactivity together with the production of hydrogenated products decreased, at the opposite the chemical yield in BFA was important again, 80%, and in parallel the radiochemical yield in BFA increased until 13%, and finally the specific activity obtained was 2.2 Ci/mMole. In this case, the reaction mixture was more complex; 8-10 products were detected by radiochemical analyses. In the experiments with a lower ratio, the formation of saturated compounds was still important; isomerized products were also observed.

Air/T ₂	Chemical Yield	Total Radioactivity	Radiochemical Yield	Specific Activity
	(BFA)		(BFA)	(BFA)
	(%)	(mCi)	(%)	(Ci/mMoles)
0	70	20	3.4	0.1
0.3	30	400	1.8	3.4
1	< 5	1500	/	(b)
2	< 5	1500	/	(b)
3	40	713	5	15
4	80	120	13	2.2
4 (a)	65	32	3.6	0.3

Table 1 : Isotopic exchange on Pd/ Diatomaceous Earth
Catalyst weight: 10.5 mg., BFA: 1.8 mg.
Room temperature, Pressure 0.25 atm., Reaction time 6 hours
(a): Argon instead of air (b): Specific activity not determined.

In this latter type of reaction conditions, i.e. air/T₂ ≈ 4, the reaction time was increased up to 36 hours. The specific activity increases rapidly to reach a quite stable value of 2.8 Ci/mMole (fig.2). After 36 hours of reaction the chemical yield in BFA was equivalent to the value observed after 6 hours, 80%, but the quantity of labelled BFA was a slightly greater: 18.5 %. The radiochemical analysis of the crude exchanged product is given in figure 3a (see Experimental Part).

In another experiment, instead of air, argon was added to the gas phase keeping the same ratio (Ar/T₂ ≈ 4). In this case, the reaction mixture was still complex and the chemical yield in BFA was about 72%. But, as indicated in Table 1, the radiochemical yield was lower and led to a lower specific activity: 0.3 Ci/mMole.

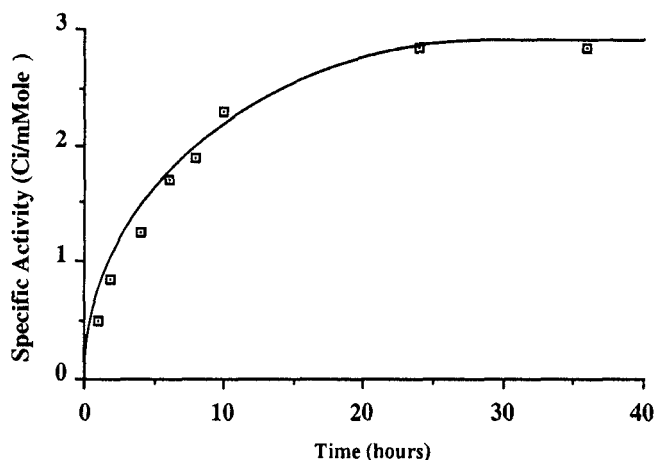


Figure 2: Evolution of the specific activity versus time of reaction
(Catalyst: Pd/diatomaceous earth)

In Table 2, the results concerning the study of Pd(II) oxide are reported. The chemical yield of BFA was low, even when the catalyst weight was reduced to 2.25 mg. The BFA radiochemical yield was also very low (specific radioactivity: 3.2 Ci/mMole). Moreover, the distribution of the reaction products presented great differences with respect to the distribution obtained on Pd supported on diatomaceous earth (fig. 3b in comparison with fig. 3a).

Air/T ₂	Chemical Yield	Total Radioactivity	Radiochemical Yield	Specific Activity
	(BFA)		(BFA)	(BFA)
	(%)	(mCi)	(%)	(Ci/mMole)
4 (a)	< 5	1320	2.4	(c)
4 (b)	30	120	5.6	3.2

Table 2 : Isotopic Exchange on Pd (II) Oxide
Catalyst weight: (a) 10.5 mg. (b) 2.25 mg., BFA: 1.8 mg.
Room temperature, Pressure 0.25 atm., Reaction time 6 hours
(c) Specific activity not determined.

III DISCUSSION.

Our results show clearly that the addition of air in the gas phase totally changes the behaviour of the catalyst. When the reaction was performed with an air/T₂ ratio about 4, the exchange was considerably enhanced and simultaneously several by-products, especially

isomerized products, were also formed. On the other hand, the proportion of hydrogenated products was lower. In order to explain these results, the decrease in the T₂ partial pressure, consecutive to the air addition could first be involved. In the case of hydrogenation and exchange reaction, hydrogen pressure effects lead to an increase of exchange (9) with respect to hydrogenation, and also to an increase of multiple exchange with respect to simple exchange (10), when the pressure is decreased. However, when argon was added instead of air, keeping the same ratio and therefore the same T₂ partial pressure, the results were different. A slight increase in exchange was observed, with respect to experiment with pure T₂ in the gas phase, but tritium incorporation was lower, indicating a lower catalytic activity. So, if there is certainly an effect of the partial pressure, this explanation is not sufficient.

When the proportion of air in the gas phase was increased step by step, air/T₂ ratio from 0 to 2, the catalytic activity, mainly hydrogenation, increased too. For a ratio equal to 2, the total hydrogenation product (both double bond) was obtained with a radiochemical purity of more than 90%. ³H NMR and Mass Spectra analyses showed that tritium incorporation was important; up to seven tritium atoms per molecule were incorporated. This could indicate that hydrogenation and exchange occurred on the same catalytic sites. Labelled BFA was only observed in very small quantity. At higher oxygen proportion, the reverse evolution was observed and finally led to the result reported above.

So, at the beginning, air addition led to an increase of the catalytic activity, as often reported for other catalytic reactions. When the air amount increased, exchange was enhanced, due to the decrease of T₂ partial pressure which limited the hydrogenation reaction. Secondly, oxygen could have played different roles as explained below and we could assume that nitrogen had not such effects. Oxygen adsorption on the metal could have modified either geometric or electronic properties of the metal particles and therefore led to the formation of specific catalytic sites on which exchange was enhanced. Moreover, if the fraction of the catalytic sites covered by O or OT (perhaps T₂O) was high, then adsorption of reactive species may have been hindered, as shown by the important increase in BFA yield. As already proposed (11) the influence of oxygen and OH (or OT) species adsorbed on the metal surface could be considered through either steric or long-range electronic effects on the environment of an adsorbed BFA molecule.

Concerning tritiated water, its formation on Pd is easy and therefore occurs in our system, even if we have no evidence of its presence in our reaction mixture. Since the exchange reaction, between BFA and T₂O did not provide good results (12), we have no indication, according to our results, of the role that T₂O could play in the catalytic mechanism.

In such a case, using Pd(II) oxide, we could expect to obtain some interesting results. However, we observed a different behaviour, and the product distribution was showing great differences, even if the reaction time was strongly decreased. In the radiochemical analysis of a sample when drawing off the reaction mixture after one hour of reaction, we observed the same result as after 6 hours. Exchange reaction was already performed on palladium catalysts supported on various support, Al₂O₃, SiO₂, BaSO₄ and charcoal(13). For each support tested, the results, i.e. product distribution, were totally different. So a role to the catalyst support could be attributed in this kind of exchange reaction, as already noted by Myasoedov et al.(14) and also by Evans et al.(2) working on PdO supported on BaSO₄.

BFA was labelled in α/β positions of both double bonds and there were certainly two different kinds of labelled species. Labelling on the double bond was only detected in a very small amount. Our results are in good agreement with those previously obtained in the study of but-1-ene/ D_2 exchange over Pd (15).

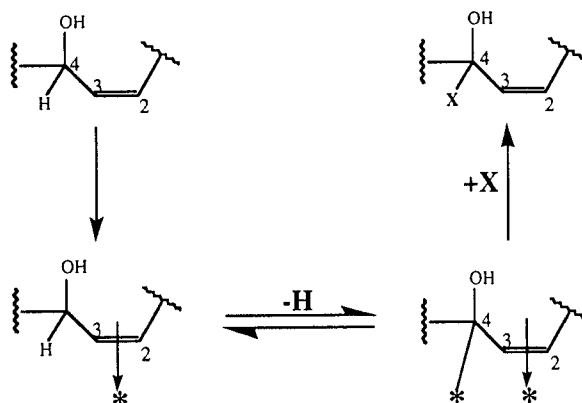


Fig. 5a

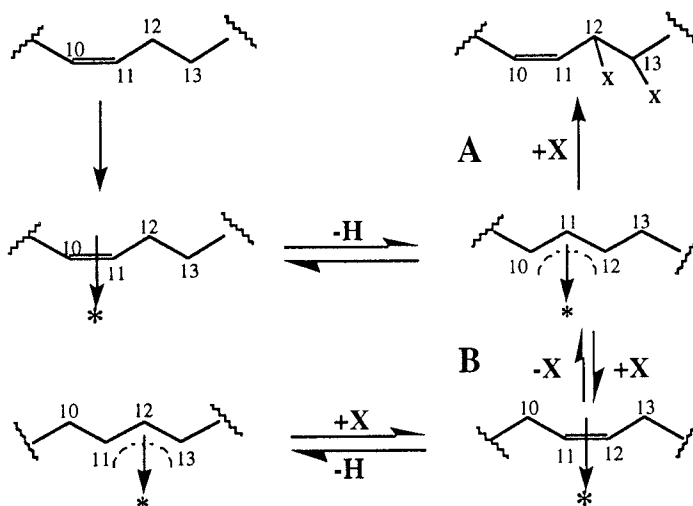


Fig. 5b

X = H or T
* : Catalytic Site

Figure 5: Possible mechanisms for tritium incorporation

We now first discuss the exchange which occurred on the carbon 4. One can suppose that the exchange proceeds through a π allylic mechanism, as first proposed by Rooney and Webb in heterogeneous catalysis (16). But in such a case, we would obtained an enol form, then this

species would turn in the ketonic form and desorb. BFA adsorbs associatively by the nearest double bond and forms a π adsorbed species. The hydroxyl group is at the opposite from the surface and therefore the hydrogen atom is close to the surface. Then, this species could turn into a " σ - π " adsorbed species, by losing an hydrogen atom as shown on fig.5a. This intermediate could desorb by a cis addition of a tritium atom to form labelled BFA without any further reaction step. The slight exchange on the double bond certainly occurred through the same reaction pathway.

BFA could also adsorb associatively by the other double bond and then could give a π allylic species (fig.5b) by losing an hydrogen atom on carbon 12. This intermediate could desorb as BFA, taken an hydrogen (tritium) adsorbed atom or be hydrogenated on carbon 10 and give a π adsorbed species with the double bond isomerized. These species could again turn into a π allylic species, and then the exchange on carbon 13 would be possible (fig.5b). All these steps are reversible and during the reverse procedure, in way B, fig. 5b, the same hydrogen (tritium) atom, as previously taken on carbon 10, would be lost. The free rotation of this carbon to put the other hydrogen atom close to the surface is impossible, due to the rigidity of the molecule. For the same reason, we suppose that only one position can be exchanged on each of the carbons 12 and 13. With a such mechanism, the formation of isomers product is easy, as observed in our results when exchange was enhanced.

Due to the isotopic dilution on the catalyst surface, by this mechanism, labelled BFA could be obtained with either only one tritium atom in position 12 or 13, or two tritium atoms in both 12 and 13 positions, using the way B. BFA labelled only in position 12 could mainly be produced by the way A, fig. 5b. By NMR, it was found that labelling was three times more important on carbon 13 than on carbon 12. Therefore, we could suppose that the proportion of molecules labelled on both position was minor, with respect to singly labelled molecules.

IV CONCLUSION.

Our results are leading to a new method of labelling. The possibility of labelling BFA by a catalysed isotope exchange reaction is now clear. This method can certainly be used in the case of other compounds of biological interest and of course for labelling with deuterium.

The influence of air in the gas phase on the exchange reaction was also well established. Exchange is considerably enhanced when the reaction was performed with an air/T₂ ratio about four. However, the role played by oxygen in the catalytic mechanism has not yet been fully explained. Further experiments have to be done in particular to attempt to answer the following question: Does tritiated water play a role or not in the reaction? In the same way, the roles of catalyst support and of pressure, which seem important according to our results, have to be investigated more precisely.

A better understanding of the reaction mechanism will permit us to determine exactly which factors govern the exchange reaction. And therefore, we can hope to obtain better specific activities.

V EXPERIMENTAL PART.

General procedure.

Two catalysts were tested:

- i) Pd supported on diatomaceous earth, 5 % metal loading, Fluka puriss.
(ref.: 76042 (A56451); batch: 402144)
- ii) Pd(II) oxide, 78 % in Pd, Fluka puriss. (ref.: 76080; batch: 220418786)

All experiments were performed using a Toepler pump system for T₂, air or argon introduction and pressure regulation. The tritium gas was obtained in breaksealed glass ampoule of 20 Ci from Radium Chemie (Switzerland). The catalyst (10 mg) was suspended in 1,4-dioxane (1 ml) in a 10 ml reaction vessel. Before each exchange test, the catalyst sample was treated under T₂ at 0.6 atm., room temperature, for 12 hours. This first step permitted us to obtain a good reproducibility from one experiment to another. Then the solution was frozen in liquid nitrogen and the gas phase was evacuated. BFA, typically 1.8 mg in 2 ml dioxane, was added. The gas mixture, T₂, T₂/air or T₂/Ar was introduced at 0.25 atm. . The solution was stirred during 6 hours at room temperature. Following the exchange reaction, the catalyst was filtered off and labile tritium was removed by successive evaporation in vacuo of methanol. Labelled BFA, obtained using Pd on diatomaceous earth catalyst, was purified, using a preparative HPLC system (reversed phase, ODS, H₂O 75 CH₃CN 25).

Analytical procedures.

The total radioactivity was determined by liquid scintillation counting following an appropriate dilution. The reaction products were analyzed by HPLC with an UV detector (Varian 2050) and a radioactive detector (Berthold LB503, liquid scintillation). BFA was detected at its maximum absorbance at 215 nm. Labelled BFA was analyzed by HPLC simultaneously with a standard BFA solution in an apparatus equipped with UV and radioactive detections. [³H] BFA and unlabelled BFA have the same retention times using three different analytical systems (250 mm x 4.6 mm columns):

*Nucleosil ODS

Eluant: H₂O 70, CH₃CN 30

Retention Volume (BFA): 18 ml

*Zorbax CN

Eluant: hexane 85, *iso*-propanol 15

Retention Volume (BFA): 14.5 ml

*Shandon Hypercarb

Eluant: H₂O 20, CH₃OH 80

Retention Volume (BFA): 16.5 ml.

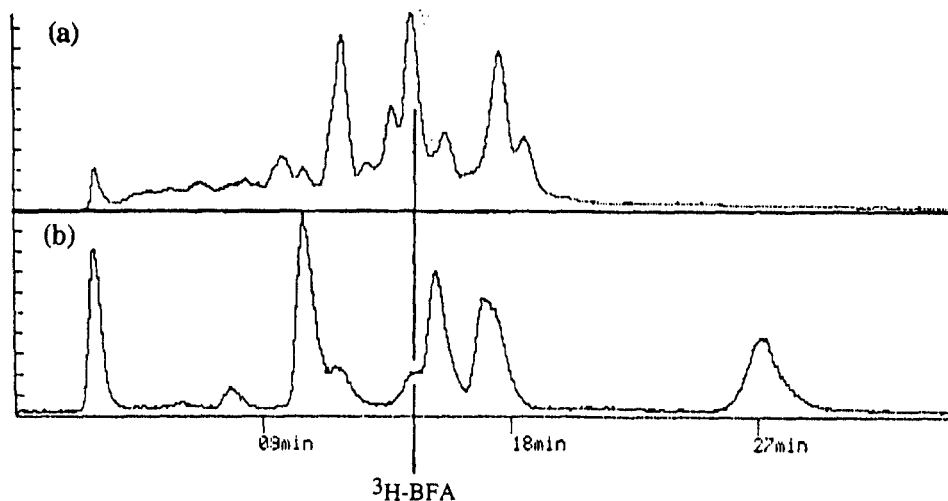


Figure 3: HPLC radiochemical analyses, air/ T_2 : 4
 Column: Zorbax CN, Eluant: hexane 85 *iso*-propanol 15
 (a) Pd on diatomaceous earth (b) Pd (II) oxide

^3H NMR spectra were recorded on a Bruker AC300 at 320 MHz. All tritium spectra were obtained with broadband proton decoupling. The solvent used for NMR samples was CD_3OD . For ^3H NMR spectra, 2 mCi of BFA (purity > 95%) were dissolved in 400 μl of CD_3OD . The spectra (fig.4) obtained was in total agreement with ^1H NMR spectra of BFA (^1H NMR spectra, interpreted using an ^1H - ^1H COSY, is not given here).

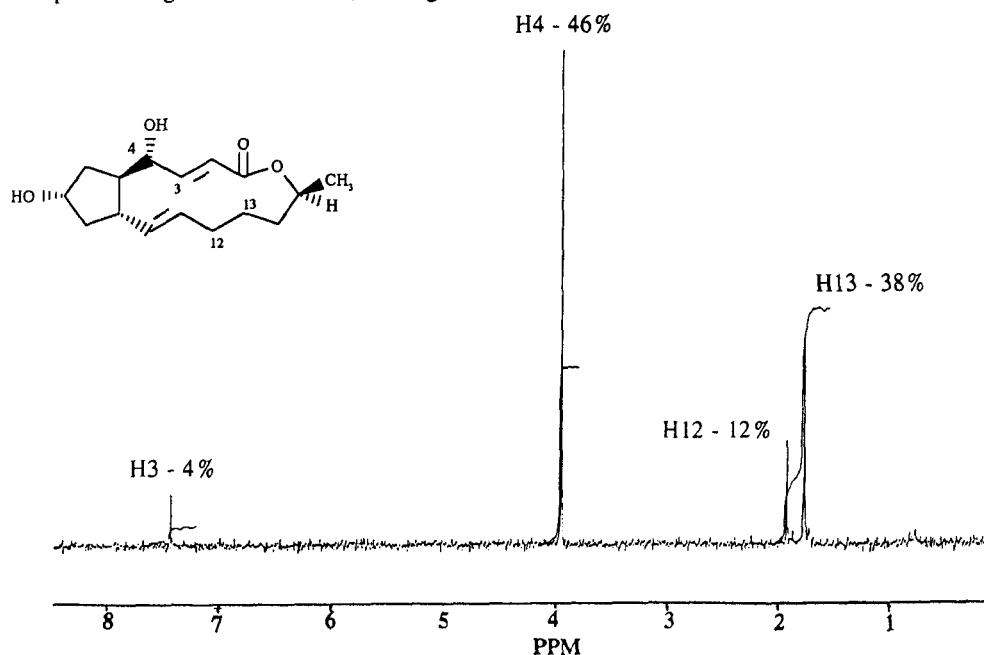


Figure 4: Labeled BFA: ^3H NMR spectra and related attribution.

The exchange occurred principally in α of both double bonds (δ : 3.9 and 1.9 ppm for carbons indexed 4 and 12 respectively, see fig.4) and in β position of the double bond in the bottom chain (δ : 1.7 ppm, carbon 13, see fig.4). Little exchange was also observed on the double bond in the upper chain.

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