

TRITIUM RECOIL LABELING OF PHENYLACETIC ACID

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SUMMARY

Phenylacetic acid has been labeled by means of the nuclear reaction ${}^6\text{Li}(n,\alpha){}^3\text{H}$, both in mixture with Li_2CO_3 and as the lithium salt. The amount of Li_2CO_3 was such as to keep the same weight ratio of lithium and phenylacetate ion as in the salt.

It was observed that intimate mixtures of phenylacetic acid and lithium carbonate react slowly indicating that previous studies on the irradiation of mixtures of organic acids with lithium carbonate have not been performed with well defined systems.

A considerably higher specific activity of the acid was found in the case of the irradiation of the lithium salt. A series of degradative reactions which insures a double determination for the activity at each molecular position was applied. In contradiction with previous results, it was observed that considerable labeling takes place at the α -position; somewhat less for the salt. The overall picture is one of uniform labeling in both cases, in agreement with the lack of selectivity shown by hot atom reactions.

INTRODUCTION

The nuclear transformation: ${}^6\text{Li}(n,\alpha){}^3\text{H}$, since the first report by Wolfgang, Rowland and Turton in 1955 [1] has been widely utilized as a means of obtaining energetic tritium atoms for the study of hot reactions and the labeling of organic molecules. The technique, in general, consists in irradiating with thermal neutrons a mixture of the organic substrate

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with a lithium salt, the carbonate in the majority of cases, and, although there is an extensive literature on the tritium labeling of organic substances in this manner [2], very few papers have dealt with the irradiation of lithium organic salts as such.

Acetic [3] and benzoic [4,5] acids have been irradiated in the form of their lithium salts. In the case of lithium benzoate, the Russian authors studied the tritium yield in the form of H_2 and C_6H_6 , which turned out to be very different from the mixture case, but they did not determine the label distribution in the parent molecule. The Japanese workers, on the other hand, reported a surprisingly high chemical yield of 99% for the lithium benzoate in contrast to only 45% for the mixture, and also a much higher specific activity, while the distribution was approximately the same in both cases. A different but relevant study is the bombardment with 40 eV HT^+ ions of targets consisting of sodium benzoate and phenylacetate [6].

In this paper the comparative results of the irradiation of phenylacetic acid mixed with lithium carbonate and of lithium phenylacetate are examined. Phenylacetic acid was considered a suitable substrate since it has both aromatic and aliphatic hydrogens and its irradiation by the mixture technique has already been studied [7].

EXPERIMENTAL

Materials. Pure commercial phenylacetic acid was repeatedly recrystallized from water and from hexane, and its purity checked by thin layer and vapor phase chromatography. From this purified acid, the lithium salt was prepared by neutralization with analytical grade lithium hydroxide and recrystallized by concentration from methanol since solubility does not increase appreciably with temperature.

Samples and irradiation. All samples weighed around 1 g and were degassed and sealed under vacuum in quartz ampoules. Irradiations were performed in the thermal column of the 1 Megawatt pool type research reactor in Mayagüez, Puerto Rico, with a flux of $2.0 \times 10^{12} \text{ ns}^{-1} \text{ cm}^{-2}$ for 2 hours. Lithium phenylacetate was irradiated as such, while the acid was intimately mixed with 27.1% its weight of analytical grade lithium carbonate. In this manner, both types of samples contain the same percentage of lithium per weight of phenylacetate ion.

Counting. Activities were determined by means of a LS-II Beckman Liquid Scintillator. Since lithium phenylacetate can be counted in dioxane-water solution, all samples (weighing less than 20 mg and weighed to the nearest μg in a Cahn electromicrobalance) were counted for uniformity in a mixture of 0.5 ml H_2O and 15 ml dioxane scintillating solution (7g PPO, 75mg POPOP, 50g naphthalene and up to 1 l with purified dioxane). The sole exception was trifluoroacetoxy thallium phenylacetate which was dissolved in 1 ml anhydrous dimethyl sulfoxide and 14 ml of toluene based scintillating solution (5g PPO, 50 mg POPOP and toluene up to 1 l) added. Efficiencies were determined by means of a calibration against the external standard factor given by the instrument. To relate the efficiency in the case of the thallium derivative to this calibration, and internal standard was used.

Since all positional activities are determined by the algebraic sum of two or three terms, all samples were counted until a minimum of 40,000 counts was accumulated to insure acceptable statistical accuracy. The errors given in tables are standard deviations calculated on the basis of the statistical nature of radioactive decay and the laws of error propagation. Actual errors are higher. The activities reported correspond to a sample of phenylacetic acid in mixture with lithium carbonate (A) and another of lithium phenylacetate (B) for which complete analyses were performed. Partial analyses of other samples gave values in good agreement.

Activity of irradiated samples. After a "cooling" period of 3 days, 1 mg aliquots of the irradiated samples were counted. The samples were diluted with a 50 fold amount of pure phenylacetic acid, calculated on the basis of the amount present prior to irradiation, and repeatedly recrystallized from water. This treatment removes carboxyl bound tritium and tritiated impurities. Once constant activity was achieved, recrystallization was continued from heptane. No further change in activity was observed.

Table 1

	Activity of crude irradiated sample Ci/g ($\times 10^4$)	Activity of purified acid corrected by dilution factor Ci/Mol ($\times 10^2$)
A	1.327	1.349
B	1.932	1.879

Positional activities. The activity in all the positions in the molecule was determined by means of degradative, substitutive and exchange reactions. The scheme followed is shown in Fig. 1. Phenylacetic acid (I) was neutralized and oxidized in aqueous solution to benzoic acid (II) with potassium permanganate. This oxidation was also accomplished by means of chromium trioxide in acetic acid. Although in the last case the yield was very low, the same specific activity was obtained. 3,5 and 2,4-dinitrobenzoic acids (III, IV) were prepared from benzoic acid (II) and phenylacetic acid (I), respectively, by standard nitration procedures [8]. *o*-Iodophenylacetic acid (VI) was prepared via the *o*-thallium trifluoroacetate derivative (V) according to the procedures developed by McKillop, Taylor and co-workers [9]. (VI) was oxidized to *o*-iodobenzoic acid (VII) in the same manner as phenylacetic acid. *p*-Iodophenylacetic acid (VIII) was prepared by the iodination technique of Datta and Chatterjee [10], and then oxidized to *p*-iodobenzoic acid (X).

To prepare α -tritium depleted phenylacetic acid (IX) a well known exchange reaction in basic medium was applied. A report by Atkinson and collaborators [11] indicates a rather facile exchange in aqueous solution. For this work, exchange in methanol solution was attempted. One hundred milligrams of phenylacetic acid was dissolved in 50 ml of a 10% methanolic solution of sodium methoxide and refluxed for 24 hours, after which the methanol was distilled off and counted. Fresh methanol was added and the procedure re-

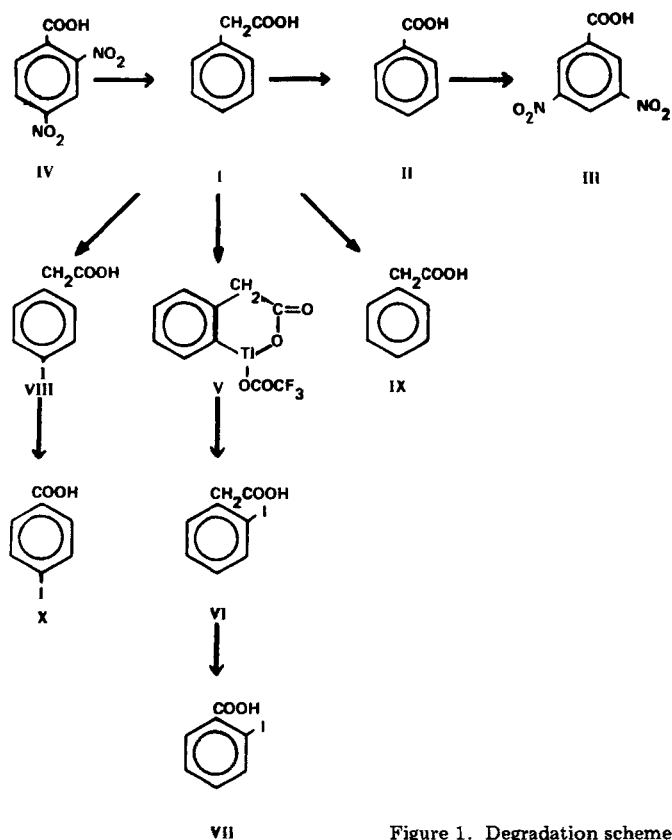


Figure 1. Degradation scheme

Table II

Molar specific activities corrected by dilution factor:
Ci/mol $\times 10^3$

	A	B
I	1.349 \pm 0.007	1.879 \pm 0.009
II	0.960 \pm 0.005	1.450 \pm 0.007
III	0.578 \pm 0.003	0.837 \pm 0.004
IV	0.582 \pm 0.003	0.879 \pm 0.004
V	1.021 \pm 0.005	1.418 \pm 0.007
VI	1.017 \pm 0.005	1.429 \pm 0.007
VII	0.782 \pm 0.004	1.167 \pm 0.006
VIII	0.983 \pm 0.005	1.371 \pm 0.007
IX	0.933 \pm 0.005	1.486 \pm 0.007
X	0.796 \pm 0.004	1.157 \pm 0.006

peated until the activity in the methanol was undistinguishable from the background. The dry residue was taken up in water, acidified and extracted with dichloromethane. After drying the solvent was eliminated and the residue recrystallized from water.

All derivatives were recrystallized until constant activity, and their purities checked by standard methods. These activities are listed in Table II. The relationship utilized for the calculation of positional activities and the corresponding results are indicated in Table III.

Table III
Percentage positional activities in terms of individual hydrogen atoms

Position	Relationship	A	B
α	$\frac{(A_I - A_{II}) \times 100}{2 A_I}$	14.4±0.3	11.4±0.3
α	$\frac{(A_I - A_{IX}) \times 100}{2 A_I}$	15.4±0.3	10.5±0.3
o^-	$\frac{(A_{II} - A_{VII}) \times 100}{2 A_I}$	13.2±0.5	15.1±0.5
o^-	$\frac{(A_{III} + A_{IV} - 2 A_{II}) \times 100}{2 A_I}$	14.8±0.5	14.2±0.5
m^-	$\frac{(2 A_{VII} + A_X - 2 A_{II}) \times 100}{2 A_I}$	16.3±0.5	15.7±0.5
m^-	$\frac{(A_{II} - A_{III}) \times 100}{2 A_I}$	14.2±0.5	16.3±0.5
p^-	$\frac{(A_{II} - A_X) \times 100}{A_I}$	12.2±0.5	15.6±0.5
p^-	$\frac{(2 A_{II} - A_{III} - 2 A_{IV}) \times 100}{A_I}$	13.2±0.5	16.2±0.5

DISCUSSION AND CONCLUSIONS

From the values for the activity of the purified acid, it is clear that hot tritium entry is considerably higher in the case of the lithium salt.

Assuming that there is no neutron flux attenuation with depth, it can be shown that 100% entry would lead to a specific molar activity in Ci/mol given by the following expression:

$$a = 0.204 \times 10^{-17} \phi t$$

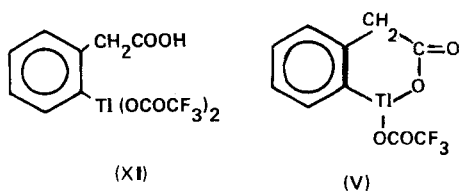
where ϕ is the neutron flux and t the irradiation time in our experiment, $2 \times 10^{12} \text{ ns}^{-1} \text{ cm}^{-2}$, and 7200s, respectively, leading to a maximum possible activity of $2.94 \times 10^{-2} \text{ Ci/mol}$. For our mixture of lithium carbonate and phenylacetic acid, the same activity would be expected.

ted since the ratio between the weight of lithium and the weight of phenylacetate is the same as in the case of the salt. Thus, entry would be 64% for lithium phenylacetate and 46% for the mixture. In view of the uncertainty in the value for the neutron flux, these results should be considered as only approximate. However, their ratio is accurate and shows considerable advantage for the irradiation of the salt.

Furthermore, it was observed in the course of this work that intimate mixtures of phenylacetic acid and lithium carbonate react slowly. This fact casts a doubt on previous reports in the literature (as well as on this one) referring to the irradiation of mixtures of acids and lithium carbonate, which in all likelihood refer to ill-defined mixtures resulting from partial reaction, where one of the products, water, is probably harmful. On the basis of these considerations, it must be concluded that the irradiation of the salt should be the preferred technique.

For the determination of activities at the different positions on the ring, nitration of both phenylacetic and benzoic acid is sufficient. Nevertheless, confirmation was provided by means of additional derivatizations through iodination and thallation. The objection might be raised that in the strongly acidic media required for nitration, ring activity could be lost through exchange. However, since both reactions are instances of electrophilic aromatic substitution and since the nitronium ion is a much more powerful electrophile than the hydrogen ion, the positions susceptible to exchange are those that are effectively nitrated. On iodination phenylacetic acid in the presence of nitric acid, α -hydrogen exchange is evident. This made necessary to oxidize the lateral chain to determine the activity at the *p*-position.

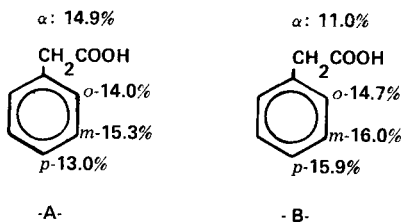
To determine the activity at the *o*-position the recent technique of aromatic thallation was applied. The derivative obtained by the thallation of phenylacetic acid in trifluoroacetic acid has already been described and assumed to be (XI), [12]. However, the results of the present study indicate that it contains only one trifluoroacetate group and should be formulated as (V)



where the oxygen bonds to the thallium atom are probably ionic. Elementary analysis for thallium gave 45.11%, in agreement with the calculated value of 45.27% for structure V. Furthermore, by utilizing the molecular weight of 451.5 which corresponds to structure V, the molar specific activities of V and VI match very closely as shown in Table II. From Table II, it is also clear than on thallation in trifluoroacetic acid, α activity is lost through exchange. Consequently, it was necessary to oxidize to the *o*-iodobenzoic acid to determine the activity at the *o*-position. In view of the loss of α activity during the acid media

reactions, we conclude that, in addition to the basic α -hydrogen exchange studied by Atkinson et.al. [11], phenylacetic acid is also capable of acid α -hydrogen exchange.

By averaging the results obtained in Table III for individual hydrogen positions, the following distributions are obtained where again A stands for the acid obtained irradiating the mixture with Li_2CO_3 , and B for the acid obtained by irradiation of the lithium salt:



The most striking difference with the results previously reported by Elatrash and Johnsen [7] is that in the present work considerable activity at the α -position is found. Lack of activity at this position was interpreted by Elatrash and Johnsen as evidence for an excitation-decomposition reaction taking place on substitution at α . Their view was that although α -substitution may take place, it will leave the molecule in an excited state which undergoes facile decarboxylation. The evidence of this work is that considerable α -substitution takes place. However, the possibility for the excitation-decarboxylation reaction is not completely excluded, but for the salt, not the acid as suggested by the mentioned authors, as the small but significant difference between the irradiated salt and the acid might be indicative that some decarboxylation takes place in the case of the salt. In connection with this, it may be mentioned that thermal decarboxylation is easier for the salt than for the acid.

A minor difference is that Elatrash and Johnsen reported the p -position as the more intensely labeled. In our corresponding experiments (A), we find, instead, less label at the p -position. With the exception of the lower activity at the α -position of the acid obtained by irradiation of the salt, it is clear that the attacking hot tritium atoms show practically no positional preference and that almost uniformly labeled products are obtained. This is in agreement with what is known in general about hot atom reactions. In view of the uncertainty of the positional results, it does not seem fruitful to discuss at this point the slight positional differences observed.

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