

## Notes on Methodology

### A note on the preparation of small quantities of labeled fatty acid chlorides

B. BORGSTRÖM and L. KRABISCH

*Department of Physiological Chemistry,  
University of Lund,  
Lund, Sweden*

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» For the synthesis of esters containing labeled fatty acids, the most convenient starting materials are the acid chlorides. However, the preparation and purification of the acid chlorides in small quantities, according to standard procedures, generally results in poor yields.

In this note, we want to call attention to a simple procedure successfully used in our laboratory for the preparation of labeled acid chlorides. The principle is based on the finding that a labeled acid rapidly equilibrates with its acid chloride.

The rate of equilibration has been studied by mixing oleic acid-1-C<sup>14</sup> with oleyl chloride and reacting the acid chloride with diethylamine at different time intervals followed by the separation of the oleyl amide from unreacted fatty acid.

One  $\mu$ mole of purified oleic acid-1-C<sup>14</sup> was transferred to 10-ml glass ampules and dissolved in 1 ml of dry, methanol-free chloroform. To this was added 630  $\mu$ moles of oleyl chloride in 1 ml chloroform. The ampules were closed and left at room temperature. At the time intervals shown in the Table, the ampules were opened and 1 ml of diethylamine was added. Thirty minutes after the addition of the diethylamine, the contents of each ampule were taken to dryness by evaporation. The residue was then dissolved in 10 ml heptane, transferred to a glass-stoppered glass tube, and 5 ml of 0.5 N sulfuric acid was added. After equilibration and separation, the lower phase was aspirated with a syringe and the heptane solution washed in a similar fashion with 5 ml water. A 1-ml sample of the heptane solution was then removed for determination of weight and radioactivity.

Free fatty acid present in the heptane solution was extracted with repeated portions of 5 ml of 0.05 N NaOH in 50% aqueous-ethanol until the radioactivity of the heptane solution remained constant. Two extractions were sufficient for this purpose.

TABLE 1. PERCENTAGE RADIOACTIVITY ADDED AS OLEIC ACID-1-C<sup>14</sup> RECOVERED AS OLEYL AMIDE

Equilibration Time	Radioactivity Recovered as Oleyl Amide
	%
<1 min	8.9
10 "	52.4
40 "	83.2
1 hr	91.0
2 "	95.8
4 "	94.2
24 "	94.4

The radioactivity was assayed in a liquid scintillation counter according to standard procedures. The results are shown in Table 1. As can be seen, under the conditions described above, the fatty acid had equilibrated with the acid chloride in 2 hr.

The incomplete recoveries of activity in the amide form most probably is due to oleic acid present in the oleyl chloride.

The method described has been used for several years in our laboratory for the synthesis of labeled fatty acid chlorides on a small scale with excellent yields.